MAY, 1958
Vol. 12, No. 5

FT food technology

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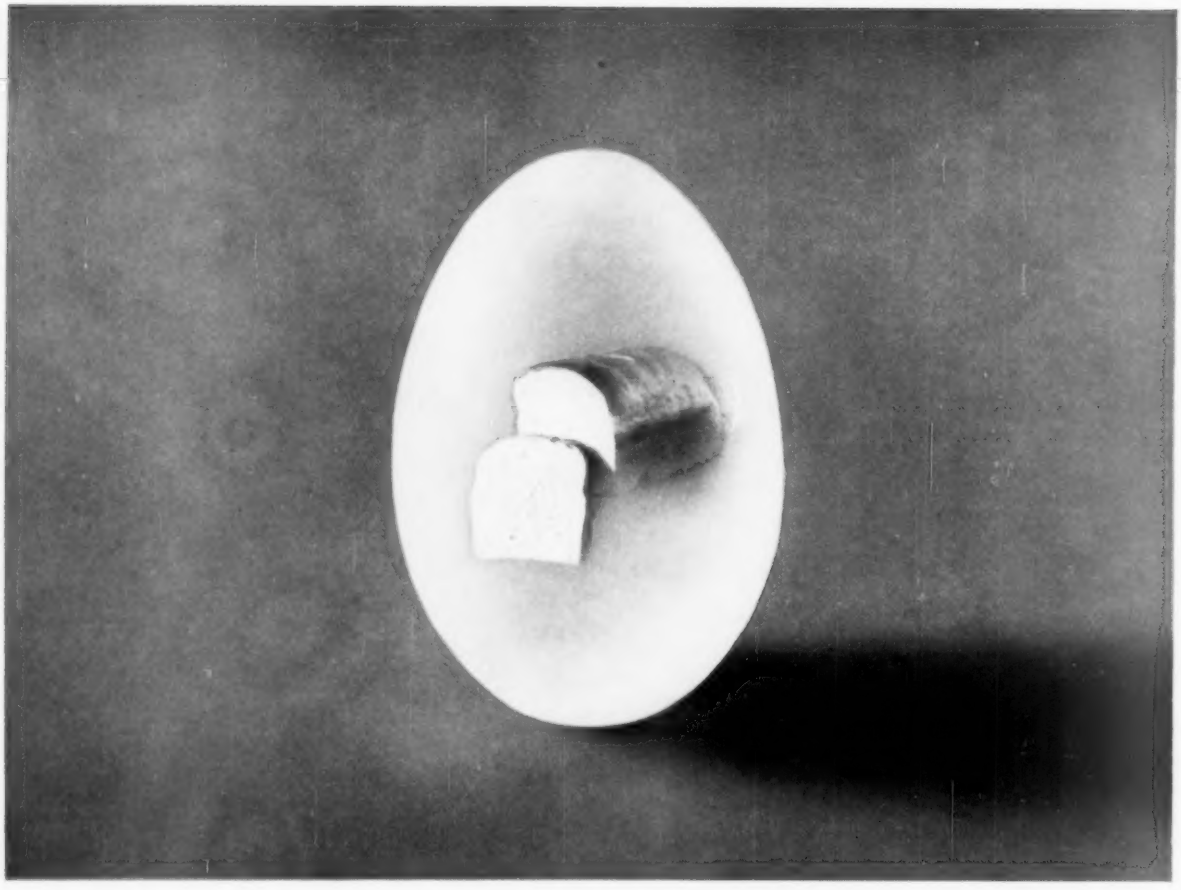
THE JOURNAL OF THE INSTITUTE OF FOOD TECHNOLOGISTS



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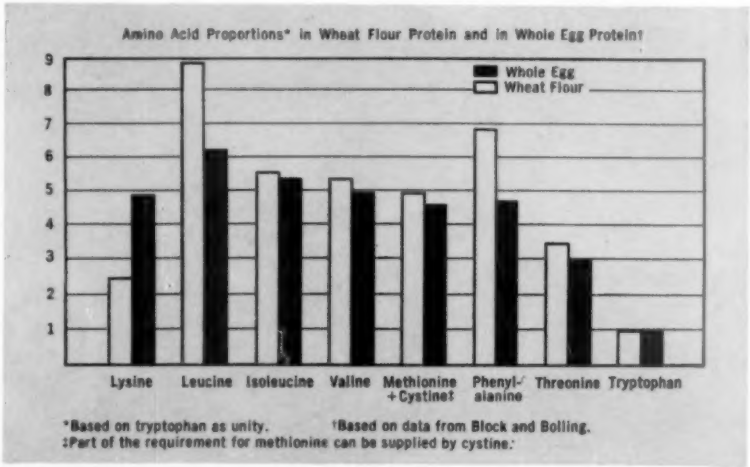

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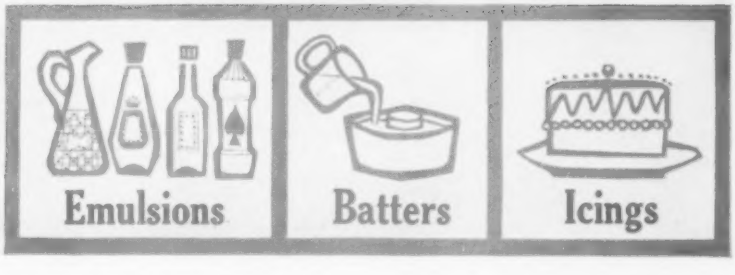

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IN THIS ISSUE—

THE TECHNICAL ARTICLES IN BRIEF

Packaging *Prepackaged meat*

Factors affecting quality of prepackaged meat. IV. Microbiological studies. A. Cultural studies on bacterial flora of fresh meat; classification by genera. Frank E. Halleck, C. Olin Ball, and Elizabeth F. Stier.

Two groups of bacteria predominated the flora of fresh meats during a four-week storage period at 34–38° F. Morphological growth characteristics of *Achromobacter-Pseudomonas* and lactobacillus colonies on the surface and in the sub-surface of a culture medium were sufficiently distinct to make possible the differentiation of the genera from each other in a mixed culture by inspection. During the first 2 weeks lactobacilli and non-pigmented organisms of the *Achromobacter-Pseudomonas* type predominated; in the later part of the storage period the *Pseudomonas fluorescens* type became dominant.

Non-pigmented *Achromobacter-Pseudomonas* constituted about 85% of the population during the first 2 weeks of storage at 34–38° F. and during the first week at 40–44° F. During the entire period lactobacilli accounted for about 5% of the population. Bacteria of the *Pseudomonas fluorescens* type constituted approximately 80% of the total count in the latter part of the storage period. In the early part they seldom exceeded 5% of the population. Packaging materials did not selectively affect rates of growth of the predominant genera.

Use of selective media to determine bacterial population shifts was unsatisfactory since the flora itself was selective. A significant mathematical correlation was found between total bacteria counts and counts obtained from the selective media. Thus, rate of increase in number of bacterial cells, which produced oxidase or lipase, or liquefied gelatin, was coordinate with the rate of increase in the total counts. (See page 197).

Fermentation *Enzyme inhibitor*

Inhibition of pectinolytic and cellulolytic enzymes in cucumber fermentations by Scuppernong grape leaves. John L. Etchells, Thomas A. Bell, and Carlos F. Williams.

Pectinolytic and cellulolytic enzymes in cucumber flowers, when added to small-scale fermentations, were effectively reduced in activity by the use of a crude extract of Scuppernong grape leaves *(Vitus rotundifolia)*. There was no apparent influence of the grape leaf inhibitor (GLI) on the character of the acid fermentation as measured by total acidity, pH, and optical density of the brine. Reduction in activities of the 2 enzyme systems was directly related to the inhibitor concentration used. Higher levels of inhibitor resulted in an increase in firmness of the fermented cucumbers (salt-stock). In general, the judges rated the salt-stock *good* to *excellent* as to acceptability for commercial use and the only comment was "darker color" for material from some of the GLI treatments. However, when salt-stock was finished into processed dill pickles and sweet pickle products, there was no appreciable difference in color of the GLI treated stock over the control. Furthermore, pickles made from all GLI lots received a quality rating of *good*. (See page 204).

Radiation preservation *Mathematical procedure for process evaluation*

Theoretical considerations in food processing by gamma radiations. II. A simplified mathematical procedure for evaluating gamma radiation processes inside hollow cylinder sources. Stanley E. Charm, Samuel A. Goldblith, and Bernard E. Proctor.

The suggested use of gamma radiation for food processing presents the problem of determining the least exposure time to yield a safe product from a microbiological standpoint. Previously described was a procedure for evaluating radiation processes by graphical determination of the dose rate distribution throughout the container and coordination of the results of such a determination with recent concepts of thermal processing. A simpler mathematical procedure has now been devised for the evaluation of gamma radiation processes inside a hollow cylinder source. (See page 209).

Wheat age *Properties of flour*

Effect of wheat age on storage properties of flour. J. A. Shellenberger, Donald Miller, E. P. Farrell, and Max Milner.

Spring and winter wheats of the 1952 and 1955 crops were compared in terms of the flours milled therefrom and bread quality. Differences in age of wheat appeared to be essentially without effect on the subsequent rate of deterioration of flours milled from the wheats studied. Spring wheat flours differed from winter wheat flours in several respects but none of the differences could be related to age. Chemical and physical evaluations, including a baking test, are reported. (See page 213).

Acceptance evaluation *Maine sardines*

A consumer survey versus panel testing for acceptance evaluation of Maine sardines. Elizabeth F. Murphy, Berton S. Clark, Ralph M. Berglund.

The effect of 5 different processing methods on the flavor of Maine canned sardines was tested organoleptically by 3 independent taste panels and by a consumer preference survey against a standard sample processed by the so-called conventional method. The 3 taste panels used the ranking technique to judge the samples: (1) whole drained fish in complete blocks of 6 samples with 3 replicates by 10 judges selected for acuity; (2) whole drained fish in balanced incomplete blocks of 3 samples with 5 replicates by 24 un-

MAY COVER. On the eve of graduation exercises at colleges from coast to coast, it is fitting to pay homage to a distinguished pioneer in the teaching of food technology and science, Dr. Samuel Gate Prescott of the Massachusetts Institute of Technology. With resolution and foresight he blazed the trail that has since become a broad avenue of learning. (Photographs, courtesy of M.I.T.)

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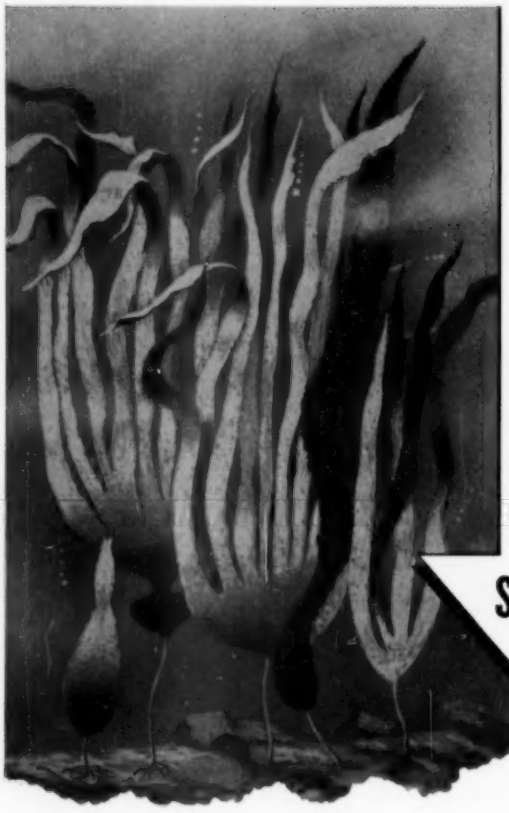

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selected judges; (3) drained, eviscerated, filleted fish cut in bite sizes in balanced incomplete blocks of 3 samples with 5 replicates by 43 experienced but unselected judges.

The consumer acceptance survey was done in 12 cities with equal numbers of families (but not always equal numbers of consumers) tasting sardines canned by the conventional method and those processed by one other method. The total number of tasters per pair of samples varied from 1000 to 1070. The consumer indicated his preference for one member of a pair and the degree of difference.

Data from the panel studies and those from the consumer survey were submitted to analyses of variance except for the data from the American Can Co. panel which were analyzed by a chi square method.

All panels and the consumers detected significant differences among the samples and all agreed that samples 59, 41, 82 and 53 (standard) were significantly preferred to samples 37 and 23. One panel only indicated that sample 82 was significantly poorer than 59, 41 and 53. The 4 highest ranking samples were retested by one taste panel (22 judges selected for acuity) by the Scheffé paired comparison method. Variance analysis of the simple preferences indicated no difference among the samples. Analysis of the degrees of difference between pairs showed that sample 59 was significantly better than sample 41 and 53. This difference was not detected by the consumer group. (See page 222).

Stability *Cured hams*

The effect of various additives on the stability of cured hams. A. M. Mullins, G. G. Kelley, and D. E. Brady.

Adding sodium ascorbate and/or sodium hexametaphosphate or calcium lactate-lactic acid mixture did not result in any marked improvement in cure penetration or uniformity in color as judged by the University of Missouri meat section staff. The outer periphery of the *semimembranosus* and *semitendinosus* muscles was generally lighter in color than the remaining portion of the center ham slice. Hams injected while hot were slightly more uniform in color than paired hams injected after being chilled.

Sodium ascorbate and/or sodium hexametaphosphate were not beneficial in preventing discoloration when these ingredients were incorporated into the curing mixture. No residual ascorbate was present in the treated hams at the end of the curing and heating process. Discoloration was delayed considerably when ham slices were sprayed with a 5% solution of sodium ascorbate or when dipped in a solution of sodium ascorbate-nitrite mixture.

There was no appreciable difference in palatability factors—i.e., saltiness, flavor, juiciness, or tenderness—in the treated hams as compared to the commercially cured or control hams as judged by the panel. Commercially cured hams and control hams ranked slightly lower on all the palatability factors except tenderness, but there was not enough difference between any of the treatments to be significant statistically. (See page 227).

Sensory testing *Difference tests*

Sensory difference tests. David R. Peryam.

This paper is concerned with certain methods which are commonly accepted as difference tests. The characteristics of difference tests are described. Test methods (the triangle, the paired, the duo-trio, the dual standard, the multiple standards, the A- not A, and the multiple pairs test) are defined and illustrated. Merits and disadvantages are discussed. (See page 231).

Antibiotics *Gulf oysters*

Chlortetracycline for preserving Gulf Oysters. A. F. Novak, E. A. Fieger, and M. E. Bailey.

Use of 20 p.p.m. CTC has merit as a preserving agent that outweighs cost for processing Gulf Oysters prior to iced or frozen storage. No disagreeable qualities appeared to develop because of inclusion of CTC. Under these circumstances the employment of CTC might be considered beneficial as it maintains consumer acceptance of the product by preventing the development of undesirable physical qualities. It is effective in low concentrations. (See page 237).

Antioxidants *Phosphates*

Protection of cooked meats with phosphates. Margaret J. Tims and Betty M. Watts.

Oxidative rancidity in cooked meat products has been measured by the 2-thiobarbituric acid reagent. Rancidity occurred in all meats tested and was not limited to those containing many polyunsaturated fatty acids. TBA values surpassed the limit of acceptability within a few hours after cooking, but such increases were not observed in raw meat.

The antioxidant affect of several phosphates salts was studied. Pyro-, tripoly-, and hexametaphosphates had a protective effect whereas orthophosphate did not. Ascorbic acid acted synergistically with phosphates to protect against rancidity in meats. All phosphates decreased cooking losses. (See page 240).

Heat inactivation *Peroxidase*

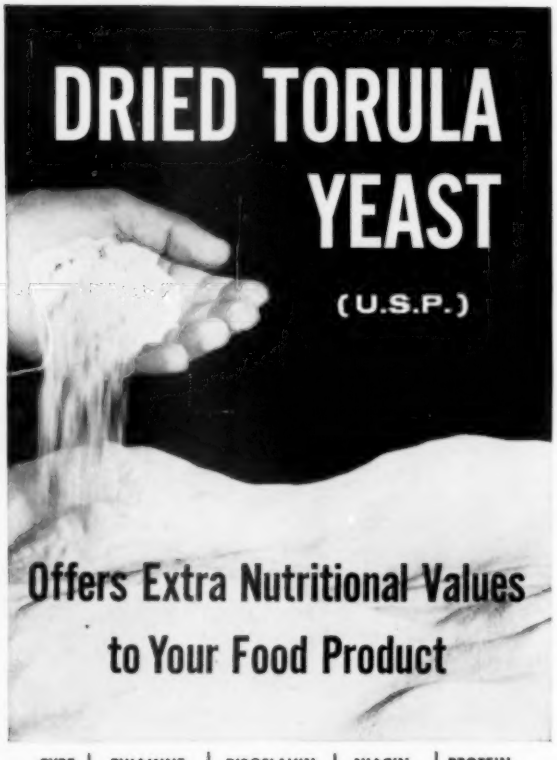
Heat inactivation of sweet corn peroxidase in the temperature range of 210° to 310° F. J. L. Vetter, A. I. Nelson, and M. P. Steinberg.

Heat inactivation of sweet corn peroxidase was found to be characterized by relatively high F_{250} and F_{300} and z values and a tendency to regenerate activity after storage. F_{250} and F_{300} values were higher when the enzyme was heated in whole kernel corn than when heated in a partially purified solution. In the case of the latter, F_{250} values found were 1.33 and 1.78 minutes before and after 72 hours storage respectively. F_{300} values were 0.22 and 0.26 minutes before and after storage. When heated in whole kernel corn, F_{250} values for the enzyme were approximately 3.7 to 7.0 minutes and F_{300} values ranged from 0.5 to 2.1 minutes depending on time and temperature of storage. Heat inactivation curves for the enzyme indicated an inflection point at about 270° F. The curves showed higher z values above this temperature. The z values found ranged from 40° F. to well over 100° F. depending on environment during heating, temperatures of inactivation, and time and temperature of storage after heating. (See page 244).

Tenderization *Papain*

Effectiveness of commercial papain in meat tenderization. Shirley Weiner, Margaret Mangel, Leta Maharg, and G. G. Kelley.

The proteolytic activity of 4 pure papains and 14 tenderizers containing papain is reported. Both milk clotting and beef digestion methods were used. The linear relationship which had been reported between the reciprocal of milk clotting time and enzyme concentration was shown to exist under the conditions of this experiment for both pure



TYPE	THIAMINE	RIBOFLAVIN	NIACIN	PROTEIN
B	0.15 mg./g.	0.05 mg./g.	0.5 mg./g.	50%
50	0.15 mg./g.	0.10 mg./g.	1.0 mg./g.	50%
90	0.27 mg./g.	0.10 mg./g.	1.0 mg./g.	50%
200	0.60 mg./g.	0.10 mg./g.	1.0 mg./g.	50%
300	0.90 mg./g.	0.10 mg./g.	1.0 mg./g.	50%

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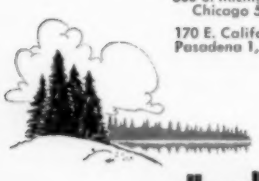

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papains and meat tenderizers. Likewise, as temperature increased the activity increased, but not enough work was done to define the relationship.

The milk clotting test was used as a standard method of assay to evaluate the beef digestion method. With the concentrations used, the milk clotting and beef digestion methods were found to give similar estimates of proteolytic activity. The activity of the tenderizers ranged from none to approximately 3% of the activity of the reference papain when tested at 40° C. The relative activity of the tenderizers was increased at higher temperatures, however. Powdered tenderizers were found to have a higher activity than most liquid tenderizers by both the milk clotting and beef digestion methods. Two modifications of the beef slurry substrate were tested but did not give more reproducible results than the slurry, although one modification was more convenient to use. Although additional work needs to be done on development of a reproducible meat substrate, it seems possible that the beef digestion method could be adapted for the study of enzymatic activity and beef tenderization. It is pointed out that there is not necessarily any relationship between the milk clotting test, the beef digestion method, and tenderness improvement as measured organoleptically. It is possible that tenderization may occur before the proteolytic effect is measurable. (See page 248).

Continuous process *Jelly*

Continuous jelly manufacture. J. D. Ponting, D. W. Sanshuck, and J. E. Brekke.

A process is described for making jellies continuously from fruit juice concentrates. This process consists in mixing metered streams of sugar sirup, pectin solution, and fruit juice concentrate. Only the sugar sirup is heated to a high temperature; therefore the fruit flavor and color are preserved by protection from heat damage. Jellies made by the continuous process retain the quality of the fruit juice concentrate used. (See page 252).

Bacteriology *Fresh meat, poultry*

Simultaneous determination of total count and fluorescent pseudomonads in fresh meat and poultry. J. H. Silliker, J. L. Shank, and H. P. Andrews.

The normal spoilage of fresh meats, poultry, and fish is due primarily to the growth of *Pseudomonas* and *Achromobacter* genera. The identification of these organisms has, in the past, been based on cultural and morphological studies made on individual isolates. Since pseudomonads are so intimately associated with the spoilage of fresh meats, poultry, and fish, a method for their direct enumeration has great value in assessing the bacteriological condition of such products. This article describes a method whereby it is possible to determine simultaneously the total bacterial count and the numbers of pseudomonads. (See page 255).

Technology *Re-use of chill tank slush ice*

Influence of re-using chill tank slush ice on market quality of eviscerated broilers. Daniel Fromm.

Under the conditions of this study the re-use of chill tank slush ice as many as 5 times did not significantly influence the bacterial numbers on chilled carcasses nor affect the shelf life of the carcasses or flavor of the cooked product. (See page 257).

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Southern Rhodesia:

Its Food Economy in Review

W. R. Carr
Food Technologist, S. Rhodesia Nutrition Council

SOUTHERN RHODESIA, a self-governing British Colony, forms the southernmost part of the Federation of Rhodesia and Nyasaland which lies entirely in the Southern tropics of Africa. The Federation is an important primary producer, particularly of copper, tobacco, asbestos and gold, of which the last three largely originate from S. Rhodesia, which is also experiencing a big new growth of secondary industry.

Southern Rhodesia covers 150,133 square miles and has a population of 193,000 Europeans, 2,350,000 Africans, 13,000 of other races. Most of the country is a plateau lying 3,000-5,000 feet above sea level, and as a consequence enjoys a sub-tropical climate with cold, sunny winters with occasional night frosts and wet summers. Agriculture is limited for the most part to one crop a year due to the concentration of the rains into the months of November to March. Rainfall varies widely not only from area to area but from season to season, in common with many tropical areas, but annual figures in the high veld areas (4,000 to 5,000 feet) are in the neighbourhood of thirty inches per annum.

A fine stand of maize.

Some indication of the rapid development of the Federation can be obtained if the national income is considered. In 1955, it was £309 million; in 1953 it was £235.7 million and for the Federal area in 1950, it was £150 million. Recent years have been noteworthy for the growth of secondary industries which are developing an appreciable export trade to other areas in Africa.

In this article, I propose to discuss the food industry of S. Rhodesia in its broadest sense, from the growing of food to the nutrition of the consumer, but first it is necessary to attempt an explanation of the ethnic situation.

Up to about 70 years ago, the African of S. Rhodesia had little contact with the white man and lived a simple primitive existence based on shifting tillage with a subsistence economy. The coming of the white man did not alter the situation for some years as there was sufficient land for the population at that time to continue with this inefficient agriculture. The white man came from the Cape Colony in the closing decade of the last century at the instance of Cecil John Rhodes, who is commemorated in the name of the Colony.

In the early days, the discovery of gold and the development of mines did much to improve communications and a railway network connected to the Union of South Africa was soon built and extended from Bulawayo in the west to the Capital City, Salisbury, where it joined the line from Umtali and the Portuguese East Africa coast at Beira. With the gold mines came farmers and ranchers and from these Europeans agriculture eventually developed. We have, therefore, in Southern Rhodesia, two distinct types of farming: the African peasant farmer, with his small plot and primitive methods and the large ranch or farm employing very often a large labour force and frequently covering many thousands of acres. The government departments covering these activities are the Department of African Agriculture, a branch of the Southern Rhodesia Department of African Affairs and the Federal Ministry of Agriculture, which is responsible for European Agriculture. There is, inevitably, considerable difference between these types of agriculture and they are best considered separately.

African Agriculture

Both the Matabele and the Mashona, the two main tribal groups who occupied Southern Rhodesia before the advent of the Europeans, lived in "extended family groups" which were (and still are) the unit of African social organization. It was a patriarchal establishment and separate huts were used by the head of the family, each wife, by unmarried sons and daughters and by dependents. Surrounding this group of huts were the gardens where maize, sorghums, millets, pumpkins and legumes such as groundnuts and cowpeas were grown. The women did most of the work in the gardens, but the men did some of the heavier work, though they took much more interest in the cattle. In former times, cattle were the source and measure of a man's wealth; they were an essential part of the marriage ceremony and they entered largely into his religious life.

Today, the picture shows vast changes and the African is no longer content with this simple life. He now

wants education for his children, a better house built of brick in place of a mud and wattle hut, agricultural implements and such consumer goods as a bicycle, European style clothing and a radio. He has gone over to a cash economy and expects to produce a surplus of grain for sale and to sell the increase of his cattle each year. This, combined with the pressure of population on the land, demanded a totally different agriculture from the traditional and in 1951 the Department of African Agriculture presented the Native Land Husbandry Act. Of its many important features are the departure from the communal system of land holding to the provisions for registration of land rights in the names of individual holders and the powers of ownership, including alienation, which this confers. The Act also includes sections on good farming practice providing for regulations over a very wide field including the prevention of soil erosion, the protection of natural resources, livestock control and farming practice generally.

The implementation of this Act is at present at full swing and is revolutionizing African agriculture with the enthusiastic co-operation of the African and the inferences will undoubtedly be far reaching. Instead of a floating population of migrant labour in the town, who come and work in the factories for a short while and then drift back to their communally held villages, the urban and rural populations will be stabilised and those owning their land will have every incentive to use better agricultural methods, with consequent far greater output, a factor which had been noted in pilot experiments.

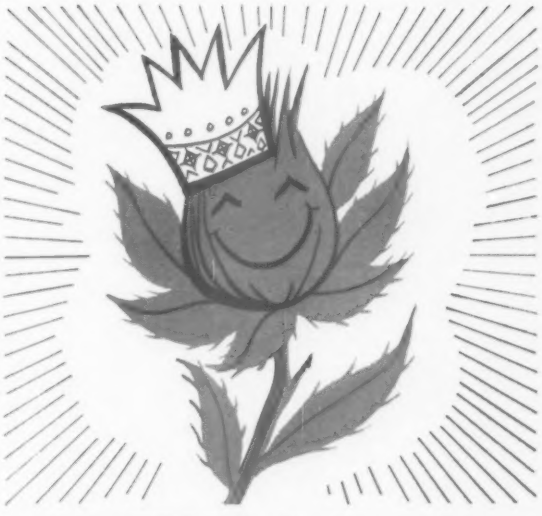
The crops grown remain fundamentally the same, but the development of vegetable gardens is taking place, with the growing of rape, cabbage, tomatoes, onions and "ladies fingers" *(Hibiscus esculentus)*. Fruit trees, such as mangoes, peaches, paw-paws, mulberries and occasionally citrus are seen near the houses, and chickens, muscovy ducks and pigeons are widely, if haphazardly, kept for home consumption.

The main cash crops at present are maize, groundnuts, sorghum, millets and beans with cotton, Turkish tobacco and castor beans in some areas. In the year ending March 31st, 1956, 700,000 bags of maize (in the year ending March, 1957, this figure has been doubled), 199,000 bags of groundnuts and over 100,000 bags of millets were sold, to give some idea of the quantities involved. The African holdings of cattle in 1956 were nearly 2,000,000 head with sales of 117,000 head.

A number of Experiment Stations are maintained by the Department of African Agriculture and are mainly concerned with the development of improved methods of farming, improvement in live stock, including development of the hardy indigenous cattle, and training of the African to the status of master farmer as well as the training of Agricultural Demonstrators who go and live with the people and help them improve their methods.

European Agriculture

One of the most striking things about the agricultural scene in the European areas of Southern Rhodesia is the extraordinary awareness among the popu-



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lation at large of the need to conserve the natural resources of the country. From an aircraft, tangible evidence of this is provided by the sight of innumerable earth dams that glitter like so many mirrors. The Natural Resources Board has been constituted to safeguard the country's natural resources and provision was made for the creation of Intensive Conservation Areas run by committees of farmers. Ninety-one of these areas have been formed and are doing valuable work in improving conservation particularly regarding erosion control.

The most important crops are as follows:

Tobacco

Virginia flue-cured tobacco is produced widely with an average yield of more than 800 lb./acre. Large Tobacco Research establishments are operated by the Rhodesia Tobacco Association and officers of the U.S. Department of Agriculture visiting Rhodesia have expressed amazement at the high level of farmer-acceptance of research findings. The auction floors in Salisbury—the largest in the world—were responsible for the exports of flue-cured tobacco in 1956 reaching nearly 140,000,000 lb.

Maize

Deliveries to the Grain Marketing Board in 1956–57 from European farms reached nearly 2.5 million bags, and the most popular variety is a white dent hybrid.

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Livestock and Dairy Farming

Ranching is widespread over large areas of the low veld, but beef production scarcely meets local demand and whilst the country is self-sufficient in milk, large quantities of other dairy products are imported. The pig population is sufficient for local requirements and a small export trade.

Other agricultural production includes tea, cotton, poultry, fruit and vegetables, and some mention must be made of the large citrus estates which run modern factories which utilise every portion of the fruit. Big developments in this field are at present being undertaken. Large sugar-cane plantations are also being developed under irrigation, but at the moment the Federation has to import most of its requirements.

Harvesting oranges at the Mazoe Citrus Estate, near Salisbury.

A number of Agriculture Experiment Stations are carrying out important rotation experiments and erosion control research. Efforts are being made to select a suitable variety of wheat, winter or summer—an important task for a country which imports 99% of its requirements. The Sabi Experiment Station is exploring the possibility of a large irrigation scheme in the Sabi River valley and full development of the proposed scheme will make a large difference to the economy of the country. The Henderson Research Station near Salisbury is running an important fisheries project and has been responsible for stocking dams all over the country and much valuable work is being carried out here to increase the productivity of the land.

Marketing and Processing of Food Crops

There are three main government sponsored bodies to aid in the orderly marketing of certain of the food crops. These are the Grain Marketing Board, who are responsible for the handling of maize and such small grain as are declared controlled products, viz., millets, sorghum, dried beans and groundnuts; the Cold Storage Commission, who handle livestock at guaranteed minimum prices to the producer, but who have, however, to enter into free competition in the buying and selling of beef; and the Dairy Marketing Board.

The Grain Marketing Board is a non-profit organisation and has by law to accept delivery of any con-

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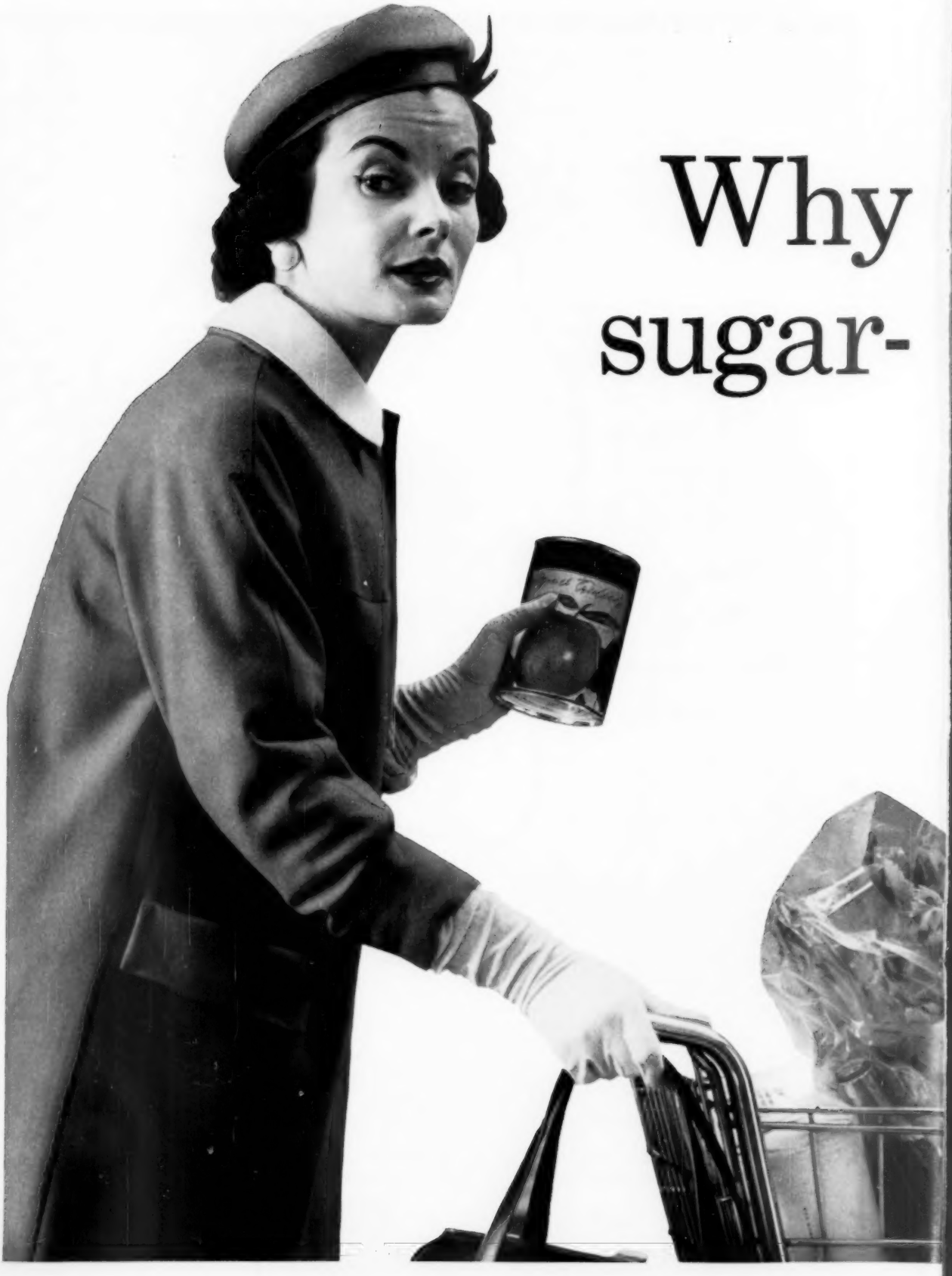

customers pick sweetened canned fruits 21 to 1*

Just because millions of people are watching their weight doesn't mean that they need, want, or will buy synthetically sweetened packs. A new super market study shows an overwhelming preference for sugar packs.

People cite better flavor as the big reason why they prefer fruits packed with sugar. There's another reason, too. Sugar's informative advertising constantly reminds people that there is no such thing as a "fattening food" or a "reducing food." The best foods to select for a weight-control diet are the same foods normally bought for the family meals.

1,579 SALES PER WEEK
in typical super market averaging $24,000 a week in volume
*75¢ gross profit per foot of shelf space**

73 SALES PER WEEK
in same type store
*29¢ gross profit per foot of shelf space**

SUGAR INFORMATION, INC.

NEW YORK 5, NEW YORK

SUGAR means sucrose (beet or cane sugar)

**Source: Progressive Grocer's "Super Valu Study." Complete study now being repeated in monthly installments in Progressive Grocer, the magazine of super marketing.*

trolled product at the time such product is offered, subject only to the proviso that it complies with certain qualitative standards. The Board is bound to carry a reserve of 6 months requirements, which makes it necessary to maintain a Pest Control Department whose operations are made more difficult by the firm hold the Khapra beetle *(Trogoderma Granarium)* has established in S. Rhodesia. Three mobile units are maintained with the necessary power sprays, plastic gas sheets, etc., and in addition the main modern silos near Salisbury are fitted with a circulating fumigation system.

The Cold Storage Commission acquires and slaughters cattle and sells beef and offals on a wholesale basis to butchers. The Pig Breeders Cooperative have recently divorced themselves from the Commission and pig products are now dealt with separately.

There are seven beef and cattle purchasing grades and the four abattoirs dealt with a total of 135,000 head in 1956 with an average C.D.W. of 466 lb. The abattoirs have been designed on modern factory lines, being housed in storied buildings thus enabling the rapid disposal of by-products with the minimum of handling.

Only chilled beef is supplied to the local market, but it is necessary to freeze a portion which cannot readily be exported in this form, hence the cold rooms are equipped for pre-coolers, chillers, freezers, dual-purpose rooms and stackers. In fact, the equipment and methods generally in use are not materially different from those in Chicago.

Weighing cans of beans, Sunrho Factory, Umtali

Modern methods are also used for slaughtering pigs and a large range of canned and cured products are manufactured after butchery into the Wiltshire type longsides or ham, Picnics, Middles, etc. A combination of wet and dry cure is used whereby sides are injected with brine under pressure, then salted and stacked. Many of the products are packed in synthetic casings, mostly imported from the U.S.A., as are the hog and sheep casings.

The Dairy Marketing Board distributes for the major part of the Dairy industry and operates seven dairies in the main centres. Pasteurised milk in bottles and "Tetrapak" cartons is mainly produced, but plain and flavoured sterilised milk will shortly be available. The sale of whole milk for the year ending June 1957 was nearly 7 million gallons, and great efforts are being made to popularise the sale of skimmed milk to the African as a protein deficient diet is only too common. A roller-drying skimmed milk plant is in operation, and Cheddar, Gouda and Roquefort cheese made.

Most of the remaining food processing firms are small, but one exception is Liebigs, who operate four factories in the country and own a ranch of 1,350,000 acres. In 1955 a number of Brahman pedigree bulls and heifers were purchased from Texas, and results so far are highly satisfactory. Products manfactured at the four factories cover a wide range, from beef and pork preparations to fruits and jams and are packed in a variety of containers. Dehydration plants are in operation, particularly in the fruit and vegetable areas of the Eastern Districts, and shortly a quick freeze plant will commence operation in this area.

Most of the Colony's wheat is obtained from Australia (90%) and the remainder is usually a strong wheat such as Manitoba. The most modern mills use pneumatic conveying for their wheat, but maize is mechanically conveyed. An 80% extraction wheat flour is milled as well as a wholemeal, a straight run maize meal prepared in either disc or hammer mills, and also a roller meal of better appearance. Negotiations are at present in progress for the enrichment of the maize meal with calcium, riboflavin and niacin, at least by the bigger millers. Maize meal is milled all over the country in small power and hand-operated hammer mills in stores, farms and villages, and enrichment control will prove difficult, if not impossible.

African Methods of Food Processing and Diet

It is unfortunate that the African is rapidly copying the worse features of the "Western Civilisation" diet, a factor which is undoubtedly causing the increasing prevalence of some deficiency diseases. This manifests itself by the purchase of aerated mineral waters, cookies, candies and buns prepared from white flour, particularly in urban areas. These products are expensive, compared with traditional foods, and, penny for penny, far less calories and protein are available.

African food stores sealed onto granite with mud.

In the villages, however, the African family is largely self-supporting as yet, and apart from taking their grain to the Store to be ground in the more developed areas, their food patterns are much the same

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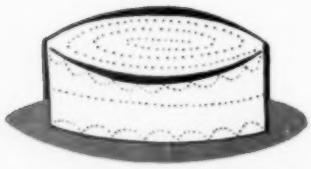

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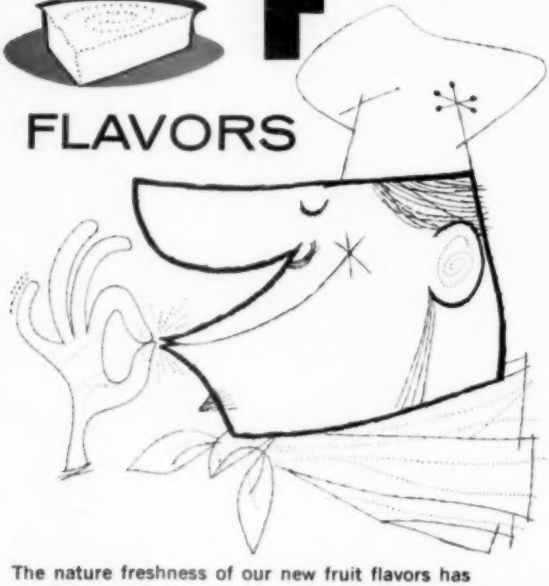

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as their fathers. After harvest, the grain and legumes are stored in huts raised off the ground or built on rock in compartments made of poles and mud that are very often completely sealed to prevent losses due to rodents, insects—and the village poultry. The traditional methods of grinding maize are remarkable in that they succeed in removing all the germ and bran and as a consequence the bulk of the B vitamins and protein, but produce a very fine white powder (yellow maize being an anathema in the Colony). The maize is dampened and broken up in a wooden mortar and then winnowed. The dehusked and degermed grits are laid in the sun to dry and then ground on the stone as illustrated, with winnowing and sieving to effectively remove any portion of the outer skin and germ of the grain which might still be there. It is perhaps fortunate from the nutritional view point that all African housewives are not so conscientious as they might be in carrying out this process. It does, of course, entail a great deal of hard physical labour. The small grains are ground directly on the stone followed by winnowing, and the meal from any of the different cereals may be cooked with water to a thick porridge, which is the staple food. This porridge is invariably eaten with a relish, which may be a meat and vegetable stew, but most often consists of peanut butter mixed with a green leafy vegetable which has been cooked for a long period of time. A description of a number of these preparations has been published *(1)*.

Other common foods are pumpkins (fruit and leaf), dried beans cooked for long periods, whole maize on or off the cob, poultry (but not eggs), sweet potato, beer, preferably made from a millet *(Eleusine coracana)* and in appearance like a thin gruel, and a large variety of wild fruits, some notes on which recently appeared in *Food Research (2)*.

The diet in the villages, given a good harvest, is reasonable, although the amount of ascorbic acid and protein in it are well below accepted standards. A bad year, however, can cause some distress, particularly in the "hungry months" from November until the new harvest. The most important deficiency disease seen is undoubtedly kwashiorkor which may be ascribed to a mixture of ignorance and poverty of mothers dealing with the weanling child.

Increasing industrialisation and urbanisation of the African, with a stable rural population will bring new nutritional dangers, particularly with the importation of new processed foods and beverages from Europe and the United States, but at the same time the per capita income should rise, as the completion in 1960 of the huge Kariba hydroelectric scheme of the Zambesi will provide cheap power for more secondary industries and the production of nitrogenous fertilisers, resulting in higher production from the towns and farms.

Acknowledgement

I acknowledge, with thanks, the permission of the Director of Medical Services, Dr. D. M. Blair, O.B.E., to publish this article.

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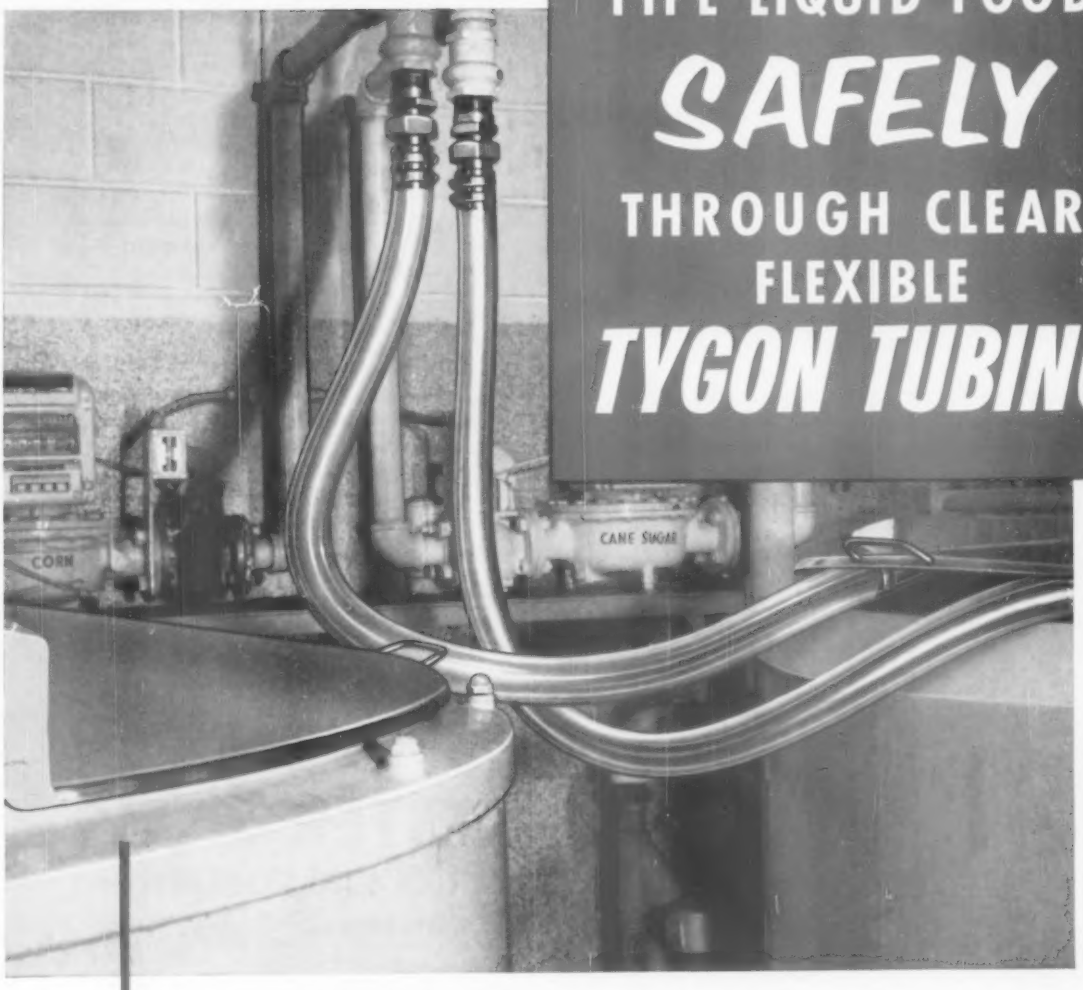

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Of Professional Interest

A Needed Additive to Food Additive Legislation

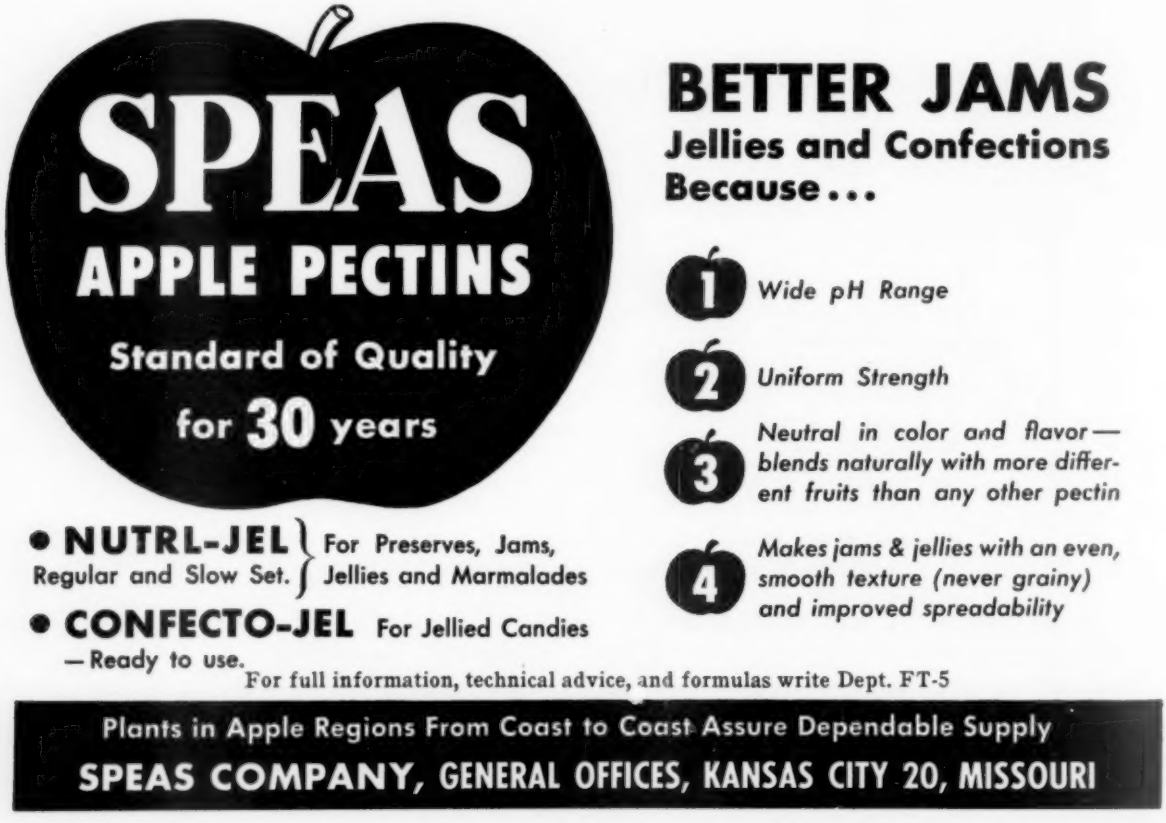
Willard E. Baier and Clarence W. Wilson

Sunkist Growers, Research Department, Ontario, California.

PROPOSED LEGISLATION is concerned mostly with new intentional food additives in prepared foods. The very proper goal is to provide for the same kind of pretesting that prevails now under the Miller Amendment in the case of incidental additives in raw agricultural commodities.

There should be a way of avoiding the very expensive reconsideration of sub-tolerance levels of approved pesticide chemicals in raw agricultural commodities when these commodities are used in processed foods. Nothing is to be gained by compounding the provisions of existing and future legislation.

We propose, in defining new additives in legislation pertaining to processed foods, wording such as the following "The term new additive does not include a pesticide chemical in or on a raw agricultural commodity as defined in Section 201 (q) of this Act; nor such a pesticide chemical present in a processed food as the result, through good manufacturing practice, of being derived from a raw agricultural commodity which complies with Section 408 of this Act provided that its established tolerance when calculated to the basis of the nonaqueous matter content of the raw agricultural commodity is not exceeded in the processed food when similarly calculated; or a food additive or treatment in use prior to January 1, 1956,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which presents no reasonable probability of injury to health; or any substance approved for use in food by or under this Act."

Difficulties otherwise encountered in translating from raw to processed foods are avoided by referring both of them to the nonaqueous matter basis. The rationale of this concept was given in earlier articles. *

It is the confident belief of the authors that some such wording as above suggested has great potentiality. Improved safety will result. At the same time conserved effort easily equivalent to millions of dollars would accrue as compared with the alternative of reconsideration of each incidental additive in all foods into which it may be carried by the raw agricultural commodities. We see no reasons for not using this simple device in proposed legislation.

* Baier, W. E. and Wilson, C. W., *Food Technol.*, **5**, 38-40 (1951) also Food, Drug, Cosmetic Law J. **9**, 170-7 (1954).

FUTURE MEETINGS FOR FOOD TECHNOLOGISTS

May 25–29	**Eighteenth Annual Meeting of the Institute of Food Technologists, Palmer House, Chicago, Illinois**
June 2–7	Eleventh Annual Industrial Microbiological Institute, Purdue University
June 8–12	Inter American Food Congress, Americana Hotel, Bal Harbor, Miami Beach, Florida. For information address J. Arthur Lewis, Box 954, South Miami 43, Florida
June 9–August 29	Gordon Research Conferences, Colby Junior College, New London, New Hampshire; New Hampton School, New Hampton; New Hampshire and Kimball Union Academy, Meriden, New Hampshire.
June 10–13	First International Congress on Vacuum Techniques, Namur, Belgium
June 12–13	First National Convention, Canadian Institute of Food Technology, Queen Elizabeth Hotel, Montreal. Theme: Canada's Food Industries
July 6–10	National Confectioners' Association 75th Annual Convention and Exposition, Sheraton-Palace Hotel, San Francisco, California
October 13–15	Association of Official Agricultural Chemists, Inc., Shoreham Hotel, Washington, D. C.
October 22–24	Fifth National Vacuum Symposium, American Vacuum Society, Sir Francis Drake Hotel, San Francisco, California

1959	
May 17–21	**Nineteenth Annual Meeting of the Institute of Food Technologists, Bellevue-Stratford Hotel, Philadelphia, Pennsylvania**
1960	
May 15–19	**Twentieth Annual Meeting of the Institute of Food Technologists, Civic Auditorium and Hotel Whitcomb, San Francisco, California**
1961	
May 7–11	**Twenty-first Annual Meeting of the Institute of Food Technologists, Hotel Statler, New York, New York**
1962	
June 17–20	**Twenty-second Annual Meeting of the Institute of Food Technologists, Americana Hotel, Bal Harbor, Miami Beach, Florida**

An open invitation is extended to readers of FOOD TECHNOLOGY to send in to the Editorial Office, 11606 South Bell Avenue, Chicago 43, Illinois notices of annual or national meetings of interest to food technologists.

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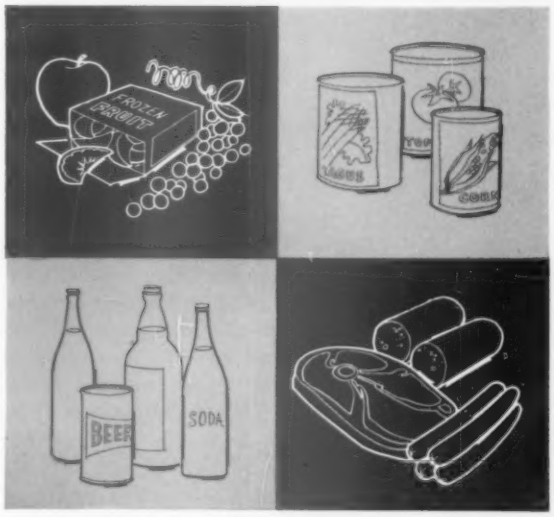

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IFT News

The Eighteenth Annual Meeting:

This Is the Month!

ARRANGEMENTS are complete for the eighteenth in the series of IFT annual meetings, and this one promises to be "the greatest" with regard to attendance, breadth of technical interest, and opportunities for professional fellowship. As usual, IFT is accenting its service to the youth of the profession, as witness the employment service, the fellowship awards, the number of papers — and good papers — being delivered by young graduates, and the number of committee appointments going to the younger members of our group. IFT has no rival in its willingness to bring youthful talents into its professional activities at the national level—and this is a healthful situation.

Emil M. Mrak

The month of an Annual Meeting is a propitious occasion to direct attention to the hard work performed during the year by the Executive Committee of the Council, the National Committees, the Editorial Boards of our journals, the National Program Committee, the National Headquarters personnel, and the host section, Chicago. The personnel of all of these groups have made invaluable contributions to the progress of IFT.

For tireless effort in guiding the destinies of IFT during 1957-58, special commendation goes to Emil M. Mrak, our president. He has travelled the length and breadth of the nation to address the Sections, to meet with IFT committees, and to coordinate our objectives with other national societies. It is not always recognized that such vigorous executiveship as has been evinced by President Mrak makes the task of keeping the Sections alive and active much easier.

This has been an outstanding year for the Institute of Food Technologists. The Eighteenth Annual Meeting will be the grand climax of a year that has contributed substantially to the professional status of the food scientist and technologist.

William F. Geddes Selected for Appert Award

Honored for Research in Cereal Chemistry

DR. WILLIAM F. GEDDES, Chief of the Department of Agricultural Chemistry at the University of Minnesota, has been named winner of the 1958 Nicholas Appert Award. This honor is bestowed annually on an outstanding figure in the food field "for distinguished contributions to the profession of food technology."

Dr. W. F. Geddes, Professor and Chief of the Division of Agricultural Biochemisty, University of Minnesota, St. Paul.

Dr. Geddes will receive the award of $1000 and a bronze medal at a special awards dinner on May 27 in Chicago during the 18th annual meeting of the Institute of Food Technologists.

The recipient has had an active career in the fields of cereal chemistry and agricultural biochemistry for nearly 40 years. A native of Manotick, Ontario, he attended the University of Toronto and the University of Minnesota. He has been affiliated with the latter university for 20 years and has served as Chief of his Department since 1944.

Through his research, Dr. Geddes has contributed significantly to the production and improvement of cereal products in the United States and Canada. Especially outstanding has been his work on the role of proteins, enzymes, and carbohydrates in the milling and baking properties of wheat, and on methods for the evaluation of flours.

More Food Processors everywhere are using Morton '999' Salt

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He has been editor of *Cereal Chemistry* magazine since 1943, and is credited with more than 220 technical papers in his field. A past president of the American Association of Cereal Chemists, he has represented the Association at international meetings in Denmark and Germany.

In 1930, Dr. Geddes was awarded the Coronation Medal of King George VI for outstanding work in his field. He also was honored by the American Association of Cereal Chemists in 1950 as the winner of its Thomas Burr Osborne Medal.

His services to the Canadian and United States governments have included five years as Chief Chemist for the Dominion Grain Laboratory at Winnipeg, Manitoba, and membership on two present committees of the National Research Council. He also is a past chairman of the Subcommittee on Cereals and Baked Products for the Quartermaster Research and Development Advisory Board.

The Nicholas Appert Award, established in 1941 by the Chicago Section of the Institute of Food Technologists, is now sponsored by the national organization. It commemorates the work of the French inventor Appert who discovered the process of food preservation by the application of heat in sealed containers.

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New York Scientist to Receive Babcock-Hart Award for Nutritional Research

A New York scientist, Dr. Bernard L. Oser, will receive the 1958 Babcock-Hart Award of the Nutrition Foundation, Inc., for his technological services to nutrition and public health. The award of $1000 will be presented on May 27 in Chicago at the 18th annual meeting of the Institute of Food Technologists.

Bernard L. Oser

Dr. Oser is President and Director of the Food and Drug Research Laboratories, Inc., of Maspeth, New York, N. Y. He was born in Philadelphia, Pa., and attended the University of Pennsylvania and Fordham University. Through his work as a research administrator and consultant he has been active in the fields of nutrition, vitamin methodology, and physiological chemistry. His special interests are in the fortification and stabilization of foods with vitamins, and in the acceptability of food additives.

He has served on numerous government committees dealing with nutrition, his most recent appointment being to a United Nations Commission investigating protein deficiencies in underprivileged countries of the world.

A Charter Member of IFT

Dr. Oser is a charter professional member of IFT and a former member of the International Committee for Vitamin Assays, the U. S. Pharmacopoeia Committee, and the board of the American Association of Clinical Chemists. He is a past president of the Council of Independent Laboratories, an associate fellow of the New York Academy of Medicine, and a fellow of the American Association for the Advancement of Science, American Institute of Chemists, and the New York Academy of Science.

He has traveled extensively in Europe as consultant to foreign drug companies and as a delegate to international meetings.

Dr. Oser will be the eleventh recipient of the Babcock-Hart Award. The award has been granted each year since 1948 by the Nutrition Foundation, Inc., in cooperation with the Institute of Food Technologists. It is given in honor of Stephen M. Babcock and Edwin Bret Hart, lifelong colleagues in nutritional research.

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Fellowships for Advanced Work Go to High Calibre Candidates

ALREADY PRODUCTIVE in research areas as complicated and challenging as radiation dose distribution; high-temperature, short time processing (a mathematical jungle for the uninitiated); and the nature and functions of an enzyme that hydrolyzes inulin, the winners of the 1958-59 fellowships for advanced work in food science are worthy of the vote of confidence given them by the Awards Committee. The competition, as in former years, was close—which attests to the high quality of students being attracted to the science and technology of food.

Robert W. Dean

Winners of the General Foods Fellowships, 1958-59

Named the winning candidates for the General Foods Fellowship were: Robert W. Dean, who has been accepted for an advanced degree by the Graduate School and the Department of Food Science, Rutgers University, New Brunswick, New Jersey; Daniel Frederick Farkas, candidate for a doctorate in the Department of Food Technology, Cambridge; and Harry E. Snyder, candidate for a Ph.D. at the University of California, Davis. Mr. Snyder, who is taking his advanced work in the Department of Food Technology, was awarded a renewal of the fellowship granted him for 1957-58.

Daniel F. Farkas

Harry E. Snyder

Arnold I. Epstein

Florasynth Fellowship Award for 1958-59

Arnold I. Epstein, graduate student at Rutgers University, Department of Food Science, has been named Florasynth Fellow for 1958-59. Mr. Epstein holds an M.S. degree (1957) from Rutgers University and is presently a candidate for a Ph.D. at that institution.

Samuel Cate Prescott Fellowship for 1958-59

The Samuel Cate Prescott Fellowship for 1958-59 was won by Kun Hyung Chang, Department of Food Technology, University of Massachusetts, Amherst. Further information on Mr. Chang was not available at the time of going to press.

Forsythe Named for Poultry Achievement Award

Dr. Richard H. Forsythe, of Springfield, Mo., has been cited by the American Poultry Institute for his contributions to poultry products technology. He will receive the A.P.I. 1958 achievement award at a special awards dinner on May 27 in Chicago in conjunction with the 18th annual meeting of the Institute of Food Technologists. The award carries with it an honorarium of $1000.

Dr. R. H. Forsythe, Director of Laboratories, Henningsen, Inc., Springfield, Missouri.

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Dr. Forsythe has been Director of the Central Laboratories of Henningsen, Inc. at Springfield since 1953. He has had a diversified career in poultry technology, as a research administrator, industrial scientist, and teacher. As an associate editor of FOOD TECHNOLOGY and as an author he has contributed generously to the professional progress of his chosen field.

He was born in Red Oaks, Iowa, and educated in chemistry and biochemistry at Iowa State College. From 1948 to 1951 he was affiliated with the Poultry Department at Iowa State, first as a graduate student and then as Assistant Professor. He later served as Associate Director of Food Research with Armour and Company in Chicago.

Dr. Forsythe's research was largely responsible for the increased use of whole egg and egg products in prepared cake mixes, icings, and meringues. He also developed special blends of whole eggs and egg yolks with sugar, corn syrups, and dextrin products which increased the storage life of these products to the point where they could be used commercially by bakers.

As a member of the Research Council of the Institute of American Poultry Industries since 1951, Dr. Forsythe has organized and participated in several industry studies toward the improvement of poultry technology. Through one of these projects an industry-wide method was adopted for determining yolk color in egg products.

French Scientist Cited for International Award

A French scientist, Henri Cheftel, has been selected to receive the 1958 International Award of the Institute of Food Technologists. Mr. Cheftel is Manager of the Research Laboratory of Ets. J. J. Carnaud et Forges de Basse-Indre, the principal container manufacturing firm in France.

The award will be presented on May 27 in Chicago at a special awards dinner held in conjunction with the 18th annual meeting of the Institute of Food Technologists. It is bestowed annually by the Australian Sections of the Institute for "outstanding efforts to promote the international exchange of ideas in the field of food technology, thus contributing to international understanding."

Mr. Cheftel has been professionally affiliated with the Permanent International Committee on Canned Foods (Comite International Permanent de la Conserve) for many years and is now Chairman of its Scientific Committee. This organization has sponsored three International Congresses on Canned Foods, at Paris in 1937 and 1951 and at Rome in 1956.

In his research work on packaged foods, Mr. Cheftel has specialized in the fields of bacteriology, nutrition,

Colleges and Universities Offering Training Programs in Food Technology (Exclusive of Dairy or Other Specialized Programs)

(As of February 1958)

Institution	*Place*	*Program Location*	*Dept. or Group Supervising Program*	*Leader of Program*
Agricultural & Mechanical College of Texas	College Station, Texas	Dairy	Committee	A. V. Moore
Cornell University	Ithaca, N. Y.	Dairy	Dairy	E. A. Nebeskey
Florida State University	Tallahassee, Fla.	Food Science & Nutrition	Food & Nutrition	Betty M.Watts
Illinois Inst. of Tech.	Chicago, Illinois	Food Engineering	Food Engineering	M. E. Parker
Iowa State College	Ames, Iowa	.....	Committee	J. C. Ayres
Massachusetts Inst. of Technology	Cambridge, Mass.	Food Technology	Food Technology	B. E. Proctor
Miami University	Coral Gables, Fla.	Food Technology	Food Technology	J. A. Lewis
Michigan State University	East Lansing, Michigan	.....	Committee	N. P. Ralston
No. Carolina State College	Raleigh, No. Carolina	Horticulture	Horticulture	I. D. Jones
Ohio State University	Columbus, Ohio	Inst. of Nutrition & Food Technology	Committee	J. B. Brown
Oregon State College	Corvallis, Oregon	Food Technology	Food Technology	N. W. Schultz
Pennsylvania State Univ.	University Park, Pa.	Horticulture	Committee	F. B. Thomas
Purdue University	West Lafeyette, Indiana	Horticulture	Horticulture	
Rutgers University	New Brunswick, N. J.	Food Science	Food Science	W. A. Maclinn
Syracuse University	Syracuse, New York	Hone Economics	Committee	Jean I. Simpson
Univ. of California	Davis, California	Food Technology	Food Technology	E. M. Mrak
Univ. of Florida	Gainesville, Florida	Food Technology & Nutrition	Food Technology & Nutrition	R. A. Dennison
Univ. of Georgia	Athens, Georgia	Food Technology	Food Technology	J. J. Powers
Univ. of Illinois	Urbana, Illinois	Food Technology	Food Technology	R. T. Milner
Univ. of Maryland	College Park, Md.	Horticulture	Horticulture	Amihud Kramer
Univ. of Massachusetts	Amherst, Massachusetts	Food Technology	Food Technology	Wm. B. Esselen
Univ. of Minnesota	St. Paul, Minn.	Agricultural Biochemistry	Committee	W. F. Geddes
Univ. of Missouri	Columbia, Missouri	.....	Committee	D. E. Brady
Univ. of Wisconsin	Madison, Wisconsin	Dairy & Food Ind.	Dairy & Food Ind.	K. G. Weckel
Virginia Polytechnic Inst.	Blacksburg, Virginia	Horticulture	Horticulture	A. Lopez
Washington State Univ.	Pullman, Washington	Horticulture	Committee	Mrs. M. M. Hard

and corrosion. He has written more than 60 papers for scientific and technical journals, and has been the principal author of a series of Bulletins published by his laboratories.

Mr. Cheftel's past honors include the Chevalier-Appert Prize, and the Prix Vernois of the French Academy of Medicine. He also is a member of the Conseil Superieur de la Recherche Agronomique and the Conseil Superieur d'Hygiene Publique de France.

Gerber Baby Foods Scholarships

Winning candidates for the six Gerber Baby Food Scholarships were announced just as we went to press. The following undergraduates were selected: Yorkette Solomon (University of Massachusetts) and Ronald Tuttle (University of California at Davis), both renewals; Robert P. Bates and Norman E. Bednarcyk of the Massachusetts Institute of Technology; Kenneth Lebermann, University of Illinois, and Gordon Wells, Jr. of Morrisville Technical Institute, Morrisville, New York. Photographs of the four new Gerber Baby Food Scholars appear below:

R. P. Bates

N. E. Bednarcyk

K. Lebermann

G. Wells, Jr.

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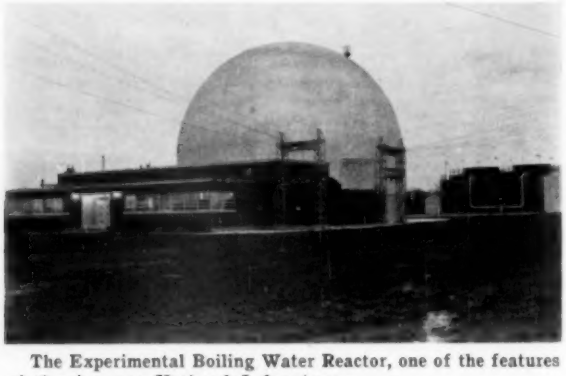
Tour Sites—Eighteenth Annual Meeting, May 26-29

Thursday, May 29, is Field Trips Day at the Chicago meeting. Pictured here are views of two sites, southwest of Chicago, where tours are scheduled; namely, the Argonne National Laboratory, near Lemont, and the Corn Products Refining Company, Argo.



The Experimental Boiling Water Reactor, one of the features of the Argonne National Laboratory.

An aerial view of the Corn Products Refining Company's plant at Argo, Illinois—reasearch laboratories in left foreground.

Canadian Breakfast

Canadians at the 18th Annual Meeting of IFT are, as in past years, planning to join in an informal breakfast which will be held on Tuesday, May 27th, Room 18, 5th floor, Palmer House. This event offers all Canadians and other interested IFT members an opportunity to renew old acquaintances, discuss informally common problems, and meet with IFT executives. Last year at Pittsburgh over 60 members from widely scattered sections of the country enjoyed each others' company at the breakfast.

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REGIONAL NEWS

S. CALIFORNIA

The name of the speaker, Dr. Lloyd Hall (Griffith Laboratories, Chicago) was certainly a drawing card for the March meeting of SCIFTS. More than 90 members taxed the assigned quarters at the Rodger Young Auditorium. Dr. Hall's handling of the subject "Technological Progress and Public Relations" belied the sentiment that technical men know little of public relations aspects of business. He stressed the importance of human relations—knowing how to get along with people—in public relations work. In addition the public relations expert must be entirely familiar with all aspects of Communication devices. He must become adept in writing, speaking, and the use of illustrations. Through the media of personal appearances, radio, television, and the public press, the housewife becomes interested in new developments in foods produced by the technological laboratory, and begins to use them. He left the thought that "The wise man of old was both philosopher and scientist, and tomorrow we may have new light on what we are sure of today." Dr. Hall stated that due to technological progress 43,000 patents relating to the foods field were registered in 1957. Program Chairman Espoy later revealed that 105 of these patents were held by Dr. Hall himself.

—Cora Miller

THE STORY OF NATURE'S YELLOW

BETA CAROTENE

WHY IS COLOR SO IMPORTANT?

You pick the big, red, beautiful apple, and every bite seems to taste better because it's so nicely red. The good, thick steak looks crusty brown, and the center's a wonderful pink. Colors call to your appetite. The best-*looking* foods seem to be the best-*tasting*. Brillat-Savarin, who wrote so well and wittily about the taste of things, said "The eyes are eaters." You don't need psychological tests to prove that foods must *look right to taste right*, but such tests *do* prove it. And your own taste buds clinch the argument.

Life would be drab without color, if everything we looked at was black and white, or some uninteresting gray. Imagine sunlight that wasn't golden, a sunset without its reds, vegetation with no green, a sky that is never blue. Our idea of beauty is based on color, on color contrast, and color harmony, at least as much as on shape and symmetry.

Some foods are naturally attractive. Some must be made so. The most enticing cooking aroma, the most delectable flavor, will be wasted if the food does not look right. Millions of oranges, picked when their skin color is an uneven greenish brown, must be dipped in liquid orange coloring solution to make them *attractive* oranges. Butter and margarine must be a good yellow for widest appeal. Gelatin desserts, children's lollipops and hard candy, soft drinks, ice cream, and many other foods must be colored for consumer acceptance. Man so often must help nature out!

Appetizing, natural-looking colors are among the best food salesmen of all. Making foods look better, making them taste better, making them nutritionally more valuable are practices everyone accepts and expects. Any improvement which allows us to enjoy more fully the gifts placed at our disposal is beneficial. Adding color is such an improvement. Food processors can now add pure Beta Carotene ROCHE*, to give natural color and vitamin A value to their good foods.

BETA CAROTENE

One of the Newer Words You See on Food Package Labels

More and more manufacturers and processors are using beta carotene—the safe, TRUE CAROTENE yellow color—to make their good foods bright, appealing, and more nutritious.

Because our federal government requires practically all food processors to declare ingredients on their package labels, you will see phrases similar to this: "Artificially colored with beta carotene." When you notice this on the labels of foods such as margarine, yellow shortening, cake mixes, and bakery products, you can be confident that this coloring, carotene, one of nature's own pigments, is safe and beneficial.

A lot of the pleasure food gives comes from the good looks *of good food. No one eats dinner in the dark. Food not seen is not enjoyed.*

*Roche—Reg. U. S. Pat. Off.

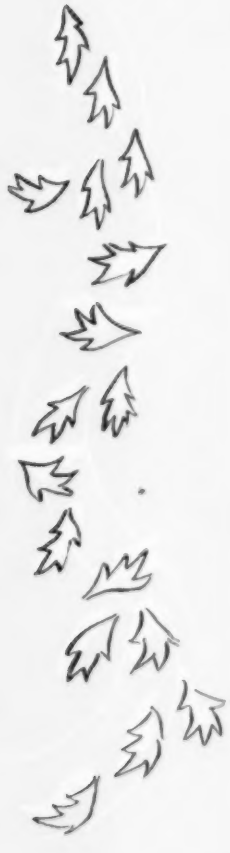
(Continued on next page)

Maybe some of you will not be too familiar with these terms used in this text:

CAROTENOID. *The carotenoids are a family of color compounds found widely distributed in nature. They account for the characteristic colors of many flowers, fruits, leaves, and vegetables. The ending "OID" is the way the chemist says: "like carotene," because all the carotenoids are similar, chemically, to beta carotene, most widely distributed, important member of the carotenoid family.*

CAROTENE, *(sometimes spelled* carotin*), is a colored compound or substance occurring in certain vegetables and fruits. In pure form its color is deep violet-red; in dilutions its color is yellow. The Greek-letter prefix,* beta, *used with the word carotene, is applied to distinguish this compound from* alpha *and* gamma *carotene—compounds which have somewhat similar chemical structure but are not nearly so important in human nutrition. In fact, the pure alpha and gamma forms are only laboratory curiosities and are not available commercially.*

Beta Carotene is a PROVITAMIN. *Provitamins are substances which are changed in the body into vitamins. The body has a mechanism by which the carotene is converted into vitamin A which we all need for good health.*



CAROTENE IS NO STRANGER

Carotene is as common as carrots, or sweet potatoes, or green leaves, or the natural yellow of butter. Carotene is the pigment which makes carrots carrot-colored and gives the orange-color to sweet potatoes. The very name carotene, of course, comes directly from the word carrot.

Carotene and other carotenoids, with chlorophyll, are present in the green leaves of plants. You see vivid proof of it when tree leaves turn yellow in fall.

THE CAROTENOIDS—A LARGE FAMILY WITH MANY RELATIONS

Nature has many pigments: the brilliant—or dull—colors of feathers, the delicious red of a ripe strawberry, the many shades of green in leaves, the blue of cornflowers, the lavender of lilacs, the differing browns of bark, the bright yellow of daffodils, the darker yellow of plump pumpkins.

The carotenoids make up one group of these pigments. They are among the most widespread of the naturally-occurring colors. Usually the reds, pinks, and yellows of many fruits, flowers, seeds and marine life, such as shellfish, are caused by carotenoids.

All of the colors in the carotenoid class have somewhat the same chemical structure. As their chemistry varies, so do their properties. Different carotenoids may exist side by side, as in some tomato species which contain beta carotene and lycopene, the so-called tomato red.

No one knows exactly how many carotenoids there are in nature. Some authorities believe the number may be as high as 200. One textbook lists 31 different carotenoids in fruits and seeds and flower petals of land plants alone.

Of all the carotenoids now known, beta carotene is the most useful for human beings, because of its value as a provitamin A. Not all carotenoids are provitamins. Alpha carotene, gamma carotene, cryptoxanthin (found in yellow corn) and some less well-known compounds have this property to only a limited extent.

For those interested in the more technical aspects of beta carotene, Roche has published a brochure which may be obtained on request.

Excellent reference works are available for the technically-minded. T. W. Goodwin's book "Carotenoids—Their Comparative Biochemistry", Chemical Publishing Co., Inc., New York, 1954, covers the functions of the carotenoids in plants and animals. "The Carotenoids" by P. Karrer and E. Jucker, Elsevier Publishing Co., London, 1950, gives a detailed account of the carotenoids. Paul Karrer, Professor of Chemistry at the University of Zurich, is noted for his work in vitamins and carotenoids, having received the Nobel Prize in Chemistry for his findings.

(Continued on next page)

BETA CAROTENE DESCRIBED

In pure form, beta carotene is deep, violet-red in color. The crystals are flat platelets in form. The pure product has very powerful coloring properties. For example, three grams of the pure crystals—enough to cover an American quarter-dollar—will color a half-ton of margarine to the accepted yellow color.

Beta carotene is soluble in various oils, vegetable oils being preferred for food use. It is insoluble in water.

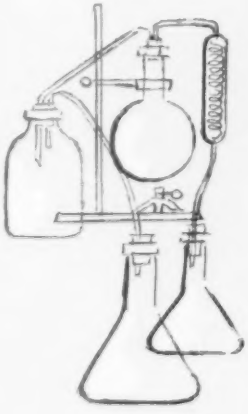
In the solutions customarily used in food processing, beta carotene Roche has practically no odor or taste, nor does it impart any odor or taste to the foods with which it is mixed.

Only a minute amount of crystalline beta carotene is needed for coloring. To simplify the measurement for manufacturers and processors, Hoffmann-La Roche has made suspensions of its finely ground crystals in vegetable oil. These are standardized, so that products with uniform color will result when they are used.

BETA CAROTENE IS A SAFE COLOR

Beta carotene is one of nature's own coloring agents. Widely present in nature, it may have first revealed itself to man when yellow-colored leaves and vegetables attracted his attention. Beta carotene has been consumed in foods for thousands upon thousands of years. Even with this evidence of safety in usage, Hoffmann-La Roche did not market its man-made "duplicate" of nature's product until careful studies showed that this substance produced no undesirable effects in test animals.

One of these tests involved the feeding of massive doses . . . so large that they were equivalent to having a 165-lb. man—every day for six months—consume 375 pounds of margarine colored with beta carotene (6,000 units of vitamin A activity per pound). Elaborate studies proved there had been no harmful effects. These and other tests indicate that beta carotene produced by the Roche process is safe and desirable for use in foods.



A CAPSULE HISTORY OF CAROTENE

Back in 1831, Wackenroder, curious to know what caused the characteristic color of carrots, isolated carotene from the roots of that vegetable.

Based on his observations, Willstätter established the empirical formula for carotene in 1906.

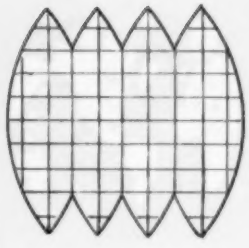
In 1919 the *vitamin A activity* of the carotenoids was discovered by Steenbock.

Between 1928 and 1930, Zechmeister, Karrer, and Kuhn established the *constitution* of carotene and assigned the structural formula.

In 1953, the successful *synthesis* of beta carotene was made by Hoffmann-La Roche. This was done by chemists who *duplicated* nature's own color as a compound in the laboratory. Their work was made easier by the preceding investigations. Pilot plant production was achieved in the same year followed by commercial production in 1954.

Thus, a period of 122 years passed between the discovery of carotene in nature and its successful duplication by Hoffmann-La Roche scientists. Carotene, however, has been used by food processors in the United States for many years. Natural extracts have been made from various sources—carrots, palm oil, alfalfa —for more than a quarter of a century. None of these natural extracts, however, has the purity of the man-made substance produced by Hoffmann-La Roche.

(Continued on next page)



HOW IS BETA CAROTENE USED?

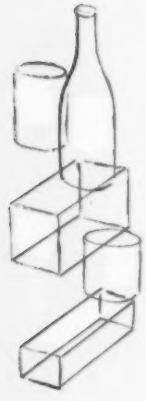
Beta carotene Roche is now used as a safe, natural yellow coloring agent for such foods as:

Margarine	*Cheese*	*Yellow Baked Products*
Shortening	*Salad and Cooking Oils*	*Beverages*
Lard	*Confections*	*Ice Cream*
Butter		

It is capable of being used in almost any processed food. Developments at Hoffmann-La Roche have made it available in oil suspensions for use in fatty foods, and in special water-dispersible forms for other foods. Other forms are in the development stage.

An acceptable coloring in the United States since 1947, beta carotene has also received official sanction in such other leading countries as Austria, Australia, Denmark, Finland, Brazil, Germany, Great Britain, The Netherlands, Norway, Switzerland.

Food processors are invited to ask Hoffmann-La Roche for technical help on the use of beta carotene Roche in their products.



WHAT ARE THE NUTRITIONAL QUALITIES OF BETA CAROTENE ROCHE?

Beta carotene is a provitamin; it is converted into vitamin A in the healthy human body.

The biological activity of beta carotene is measured in units of vitamin A activity. For instance, one gram of beta carotene equals 1,666,700 U.S.P.* units of vitamin A, while a gram of vitamin A (alcohol) itself equals 3,333,300 U.S.P. units. The U.S.P. unit and the International Unit are used for convenience in measurement. One U.S.P. unit is equal to 0.344 micrograms of vitamin A acetate.

Of the six provitamins A that are known to exist in nature, beta carotene is the most widely distributed, the most potent in vitamin A activity, and most readily available to us.

Beta carotene Roche is more than just another efficient food color. It has a nutritional benefit, too, through its ability to provide vitamin A value. As it is so intimately associated with vitamin A, it comes close to being an essential substance in itself.

WHY WE NEED VITAMIN A

We need vitamin A to guard the health of our eyes and our skin and to build our resistance to infections. People deprived of vitamin A in their diet are subject to nightblindness, which is a failure or imperfection of the vision at night or in dim light.

We need vitamin A to help maintain the health of membranes and other specialized tissues. We need vitamin A for normal growth from infancy through childhood to maturity.

According to the recommendations of the Food and Nutrition Board of the National Research Council, healthy adults in the U. S. A. should receive 5,000 U.S.P. units of vitamin A daily in their diet. The requirements for pregnancy, for infants and children, vary above and below this amount. It has been estimated that two-thirds of the vitamin A activity in the average American diet is derived from carotene and related compounds in foods. Therefore, it is important that we eat daily adequate amounts of green, leafy vegetables, carrots, fruits, as well as processed foods which have been nutritionally improved with beta carotene Roche.

**U.S.P. means United States Pharmacopeia, an official compendium of standards.*

(Continued on next page)

The Story of Nature's Yellow — Beta Carotene

OPENING ANOTHER DOOR TO THE FUTURE

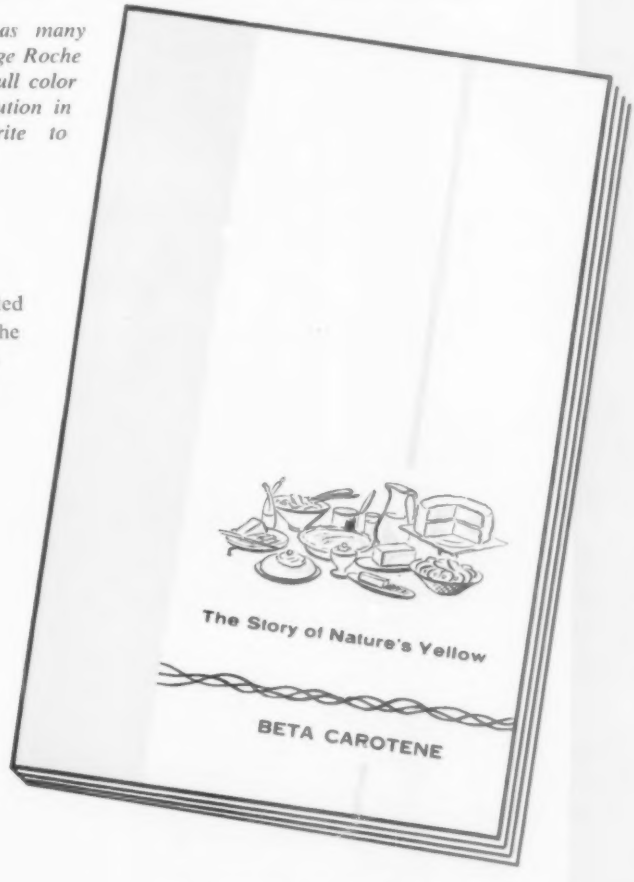
The successful synthesis of vitamin A by Roche scientists led to the discovery of the synthesis of beta carotene. The synthesis of beta carotene will no doubt lead to the manufacture of other carotenoids.

Right now, scientists are working on the processes necessary to produce other safe coloring agents of the carotenoid family. For example, work is being done on "duplicating" lycopene, the dominant red of tomatoes, and on cryptoxanthin, a yellow color found in corn.

The development of pure beta carotene Roche is really only a beginning, not an ending.

You may have as many copies of this new 20-page Roche brochure illustrated in full color as you need for distribution in your organization. Write to Dept. FT-5

Food processors are invited to ask Hoffmann-La Roche for technical help on use of beta carotene Roche in their products.



Vitamin Division

HOFFMANN-LA ROCHE INC.

Nutley 10, New Jersey

In Canada: Hoffmann-La Roche Ltd.
1956 Bourdon Street, St. Laurent, P. Q.

Canco research meets the challenge of ever-better packaging!

Using a high vacuum of considerably less than 1 micron absolute pressure, metal is vaporized and deposited as a thin film on a specimen which will later be studied under the electron microscope. This "shadow casting" technique is one of the experimental methods used at Canco's Barrington, Ill., laboratory in the long-range study of materials and methods for entirely new types of cans.

For more than fifty years, American Can Company's program of container research has been noteworthy. It was the very first venture of its kind and one of the earliest research programs sponsored by any industry.

Through the years, Canco scientists and engineers have contributed dozens of important "firsts" to canning and packaging technology. The results of their many achievements have helped millions to live better, more conveniently, for less money.

Today, in Canco's well equipped laboratories in Barrington and Maywood, Illinois, there are more than 250 scientists and technologists at work—chemical engineers, metallurgists, chemists, food technologists and many others.

Many of the greatest advances made in food packaging are a result of this program of research, the most extensive in its field today. That's why so many canners rely on Canco to keep them first with the latest container developments!

CANCO American Can Company

Factors Affecting Quality of Prepackaged Meat[a,b]

IV. Microbiological Studies. A. Cultural Studies on Bacterial Flora of Fresh Meat; Classification by Genera

Frank E. Halleck,[c] C. Olin Ball, and Elizabeth F. Stier

Department of Food Science, Rutgers University, New Brunswick, New Jersey

(Manuscript received March 20, 1956)

IN BACTERIAL FLORA of prepackaged fresh meat[d] stored at 34°–38° F., organisms of the non-pigmented *Achromobacter-Pseudomonas* type and members of the genus lactobacilli predominated during the first 2 weeks of storage and *Pseudomonas-fluorescens* type organisms during the later part of the storage period (Tables 3–11 inclusive).

Cultural studies on the bacterial flora of prepackaged fresh meats

Non-pigmented Achromobacter-Pseudomonas isolates. The *Achromobacter-Pseudomonas* type colonies have distinct morphological characteristics by which they can be differentiated from other organisms (Table 1). The colonies are characterized by the following type of colonial growth:

	Surface Colonies	*Sub-surface Colonies*
Form	Circular, large	Spindle shape, medium
Texture	Smooth	Smooth
Edge	Entire	Entire
Growth	Raised	Lenticular
Color	Tan	Tan
Lustre	Glistening	

A foul amine-like odor was produced by all non-pigmented *Achromobacter-Pseudomonas*-isolates when they were cultured on organic nitrogen media such as veal infusion agar.

Prolific growth of the isolates was found in deep culture medium which suggested that the isolates could grow under reduced oxygen tension.

Varying degrees of oxidase and lipase production by the cultures indicated that fats could be attacked both oxidatively and lipolytically. These data indicated that one of the first signs of quality degradation of the samples, exclusive of color and proteolytic changes, may be fat decomposition.

Growth of these isolates on gelatin suggested that the organisms produce proteolytic enzymes. Cultivation of the organisms on a carbohydrate-ammonium salt medium indicated that the path of amino acid decomposition may be through deamination. Growth on the carbohydrate-ammonium salt medium was enhanced by the addition of yeast extract. The cultures consistently produced a slightly acidic environment, irrespective of the nutrients supplied.

Lactobacilli. The large number of lactobacilli isolated from fresh lamb and beef (Table 2) suggest the importance of these organisms as a component of the bacterial flora of prepackaged meats.

Surface colonies of these organisms on nutrient agar were white, smooth, flat, with slightly raised centers, and undulate, translucent edges. Sub-surface colonies on nutrient agar were smooth and globose with a translucent halo around the central white growth. These characteristics were pronounced on veal infusion agar (Table 1). Visual differentiation of lactobacilli from other colonies on a culture plate was readily made. The isolates could not produce proteolytic changes or attack fats lipolytically or oxidatively. Cultures grown in glucose or sucrose broth showed that the utilization of these carbohydrates was accompanied by acid production. Other carbohydrates were not used. The function of lactobacilli in the process of degradation of proteins and fats was not ascertained.

Pseudomonas-fluorescens type isolates. Members of this genus were found abundantly on fresh meats until after 2 weeks of storage at 34–38° F. The distinguishing growth characteristics which were used to visually identify these organisms were their small, circular pinpoint appearance and the frequent diffusion of a green pigment into the culture medium (Table 1).

Flavobacterium isolates. These isolates were characterized by yellow, watery colonial growth. *Flavobacterium* colonies appeared on the culture medium only spasmodically, and, in our studies, the genus was not common to the bacterial flora of fresh meat.

Other bacterial isolates. A few colonies of the genera *Aerobacter*, *Micrococcus*, and *Bacillus* were isolated. Members of these genera are not commonly found as components of the flora of fresh meat.

Relative abundance of bacteria of various genera in prepackaged fresh meats during storage

Classification data indicated that differentiation of bacterial colonies in a mixed population on cultural medium may be made by visual inspection.

The data obtained after completion of the classification studies were characterized as follows:

1. Total bacterial count
2. Non-pigmented *Achromobacter-Pseudomonas* type count
3. Lactobacilli count
4. Pigmented *Pseudomonas-fluorescens* type count

The data in Tables 3 through 13 and in Figures 1, 2, 3 and 4 show typical bacterial growth patterns of prepackaged meat stored at either 34–38° F. or 40–44° F. The data show that the non-pigmented *Achromobacter-Pseudomonas* types are the most prominent component of the bacterial flora of the meat throughout the storage period. For the first 2 to 3 weeks of storage at 34–38° F., members of the non-pigmented *Achromobacter-Pseudomonas* group were the dominant organisms while

[a] A report of work conducted by contract with the Bureau of Animal Industry, U. S. Department of Agriculture, with funds provided under the Research and Marketing Act.

[b] A paper of the Journal Series, N. J. Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Departments of Food Science and Animal Husbandry, New Brunswick, N. J. Data from Ph.D. thesis of Frank E. Halleck.

[c] Present address: Dept. of Biological Sciences, Loyola University, Chicago, Ill.

[d] The following is the legend of codes representing the various package types used in this present investigation:

Code Number	*Packaging Material*
2	Can
4a	Vinylidene copolymer (Cry-O-Vac)
6a	MSAT80 cellophane, coated side inside
6d	MSAT80 cellophane, coated side outside
8	Cellulose acetate P-912
15	Cellophane-pliofilm laminate, pliofilm inside
17	Cellulose acetate-pliofilm laminate, pliofilm inside

in the latter part of the storage period those of the *Pseudomonas-fluorescens* type were sometimes in greater abundance. (Note: "Achromobacter, non-P" in the figures means the non-pigmented *Achromobacter-Pseudomonas* group. "*Pseu., fl.*" refers to the *Pseudomonas-fluorescens* type).

Data shown in Tables 3 through 13 and in Figures 1 through 4 indicate that packaging conditions did not affect the rates of growth of the predominant forms throughout the storage period at 34–38° F. or 40–44° F. In Run S (Tables 12 and 13), for some unknown reason, the only organisms found in the non-inoculated meat were the non-pigmented *Achromobacter-Pseudomonas* types.

TABLE 1

Cultural and staining characteristics of predominant bacteria isolated from fresh lamb and beef

Genus	Non-pigmented *Achromobacter-Pseudomonas* spp.	Lactobacilli spp.	*Pseudomonas-fluorescens* type spp.
No. Similar Isolates	96	76	25
Gram Stain	Negative	Positive	Negative
Cell Morphology	Large rods, single	Short rods, in chains	Short rods, in chains
Motility	Positive	Negative	Positive
Nutrient Agar Streak	Heavy growth, large tan glossy colonies	Scanty growth, white with raised center, and beaded. Veal infusion agar better growth	Pin point growth, glistening, green diffusable pigment
Nutrient Agar Deeps	Heavy growth, tan spindle-shape colonies. Amine-like odor	Good growth, white with translucent halo	Pin point colonies slight green pigment
Nutrient Broth	Turbid, amine-like odor	Scanty white bottom growth, butanol odor	Turbid
Gelatin Agar	Liquefaction	No liquefaction	Liquefaction
Gelatin Deeps	Liquefaction*	No liquefaction	Liquefaction*
Nitrate Agar	No reduction	No reduction	Reduction to nitrites
Glucose Broth	Acid, no gas	Acid, no gas	Acid, no gas
Sucrose Broth	Acid, no gas	Acid, no gas	Acid, no gas
Lactose Broth	No acid, no gas	Sl. acid, no gas	No acid, no gas
Glycerol Broth	No acid, no gas	Sl. acid, no gas	No acid, no gas
Sucrose Broth	Acid, no gas	Acid, no gas	Acid, no gas
Lactose Broth	No acid, no gas	Sl. acid, no gas	No acid, no gas
Glycerol Broth	No acid, no gas	Sl. acid, no gas	No acid, no gas
Litmus Milk	No reaction to sl. alkaline	Sl. acid, no curding	No reaction to sl. alkaline
Catalase Test	Positive	Negative	Positive
Starch Agar	Sl. hydrolysis	No hydrolysis	Sl. hydrolysis
Jensen-Oxidase Test	Positive*	Negative	Positive*
Turner-Lipase Test	Positive*	Negative	Positive*

Genus	*Flavobacterium* spp.	*Aerobacter* spp.	*Bacillus* spp.
No. Similar Isolates	11	5	3
Gram Stain	Negative	Negative	Positive
Cell Morphology	Large rods, single	Short rods, single	Large rods, spores
Motility	Positive	Positive	Positive
Nutrient Agar Streak	Heavy growth, yellow watery colonies, foul odor	Heavy growth, white colonies	Heavy growth, white dry colonies
Nutrient Agar Deeps	Heavy growth, large yellow colonies	Fair growth, white colonies	Heavy growth, white spreading colonies
Nutrient Broth	Turbid, yellow	Flocculent white growth	Turbid with pellicle
Gelatin Agar	Liquefaction	Slight liquefaction	Liquefaction
Gelatin Deeps	Liquefaction*	Liquefaction	Liquefaction
Nitrate Agar	No reduction	Reduction to nitrites	Reduction to nitrites
Glucose Broth	Acid, no gas	Acid, gas	Acid, no gas
Sucrose Broth	Acid, no gas	Acid, little gas	Acid, no gas
Lactose Broth	No acid, no gas	Acid, gas	Sl. acid, no gas
Glycerol Broth	No acid, no gas	M.R. Pos. V.P. Neg.	Sl. acid, no gas
Litmus Milk	No reaction to sl. alkaline	Acid, gas production, curding	No reaction
Catalase Test	Positive	Positive	Positive
Starch Agar	Sl. hydrolysis	Hydrolysis	Not tested
Jensen-Oxidase Test	Positive	Not tested	Not tested
Turner-Lipase Test	Not tested	Not tested	Not tested

* Variable activity—strong to weak.

TABLE 2

Generic distribution of organisms isolated

Genus	Number of Isolates from	
	Lamb	Beef
Non-pigmented *Achromobacter-Pseudomonas* type	52	44
Lactobacillus	28	48
Pseudomonas-fluorescens type	9	16
Flavobacterium	5	6
Aerobacter	5	0
Micrococcus	1	3
Bacillus	2	1
Yeast	1	0
Unidentified	1	2
Total	104	120

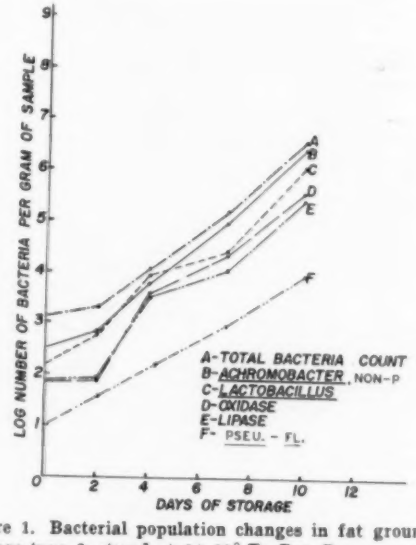


Figure 1. Bacterial population changes in fat ground lamb in package type 2, stored at 34–38° F. Run P.

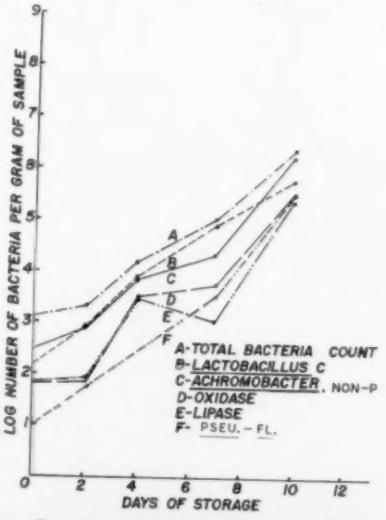


Figure 2. Bacterial population changes in fat ground lamb in package type 15, stored at 34–38° F. Run P.

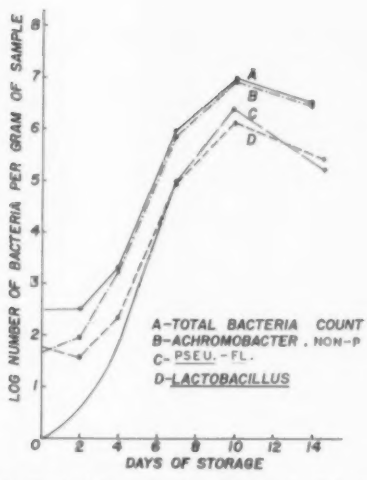


Figure 3. Bacterial population changes in fat ground lamb in package type 2, stored at 40–44° F. Run R.

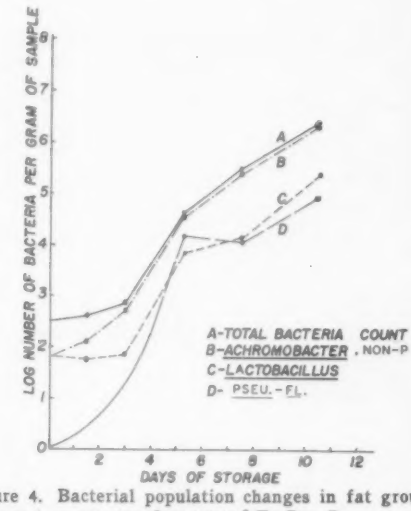


Figure 4. Bacterial population changes in fat ground lamb in package type 15, stored at 40–44° F. Run R.

TABLE 3

Mean bacterial counts per gram of fat ground pork in package types 2, 15, and 17 and stored at 34–38° F. for 37 days. Run H.

(Mean count of 6 replicates except where indicated.)

Package type	Days	Total	Non-pigmented *Achromobacter Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
2	0	6.5×10^3	5.1×10^4	.....**	.....**	4.6×10^3	3.9×10^3
	7	8.2×10^5	4.2×10^5	1.5×10^5	.....**	1.4×10^5	1.9×10^5
	14	1.4×10^6	8.1×10^5	1.2×10^5	$(6.7 \times 10^5)_1$	1.9×10^5	1.1×10^5
	21	1.2×10^6	5.8×10^5	1.4×10^5	3.4×10^5	1.2×10^5	4.1×10^4
	37	3.1×10^6	9.5×10^5	5.5×10^5	1.5×10^6	1.9×10^5	5.2×10^4
Film No. 15	7	7.7×10^5	3.3×10^5	2.2×10^5	.....	4.5×10^4	7.4×10^4
	14	2.2×10^6	9.9×10^5	3.5×10^5	1.6×10^4	9.0×10^4	5.7×10^4
	21	2.3×10^6	9.8×10^5	3.5×10^5	9.9×10^5	6.6×10^4	3.3×10^4
	37	5.6×10^6	1.8×10^6	7.1×10^5	3.3×10^6	1.4×10^5	3.6×10^4
Film No. 17	7	9.7×10^5	3.8×10^5	2.2×10^5	.....	1.1×10^5	8.9×10^4
	14	2.9×10^6	1.5×10^6	3.8×10^5*	4.1×10^5	2.5×10^5	1.5×10^5
	21	2.3×10^6	8.0×10^5	8.1×10^5	7.5×10^5	1.9×10^5	1.5×10^5
	37	4.1×10^6	1.4×10^6	1.2×10^6	1.5×10^6	3.9×10^5	2.0×10^5

* Average of 5 counts. ** None detected at dilutions plated.

TABLE 4

Mean bacterial counts per gram of fat ground lamb in package types 2, 6a, and 15 and stored at 34–38° F. for 28 days. Run N.

(Mean count of 6 replicates)

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
2	0	2.1×10^3	6.9×10^2	8.4×10^1	.....	7.9×10^1	8.0×10^1
	6	8.6×10^5	5.2×10^5	1.3×10^5	.....	2.3×10^5	1.0×10^5
	12	1.3×10^6	5.1×10^5	2.8×10^5	.....	5.5×10^4	6.0×10^4
	18	2.2×10^6	1.6×10^6	3.8×10^5	1.5×10^5	9.2×10^4	1.2×10^5
	28	8.0×10^5	4.8×10^5	1.8×10^5	1.9×10^5	6.8×10^4	6.2×10^4
Film 6a	6	3.0×10^5	2.4×10^5	7.4×10^4	.....	1.1×10^5	3.7×10^4
	12	1.6×10^6	8.4×10^5	3.5×10^5	.....	1.3×10^5	8.4×10^4
	18	4.1×10^6	3.3×10^6	6.5×10^5	1.5×10^5	5.3×10^5	3.2×10^5
	28	7.0×10^6	4.7×10^6	1.2×10^6	1.1×10^6	4.3×10^5	2.4×10^5
Film 15	6	7.3×10^4*	5.1×10^4*	1.8×10^4*	.....	9.3×10^2	1.3×10^4
	12	1.2×10^6	5.8×10^5	5.0×10^5	.....	1.9×10^4	2.3×10^4
	18	1.8×10^6	8.5×10^5	1.3×10^5	7.7×10^5	1.1×10^5	1.0×10^5
	28	3.1×10^6	2.5×10^6	3.0×10^5	3.1×10^5	3.1×10^5	2.1×10^5

* Average of 5 counts.

TABLE 5

Mean bacterial counts per gram of fat ground lamb in package type 2 that was held at room temperature for 20 hours before storage at 34–38° F. for 28 days. Run N.

(Mean count of 3 replicates)

Days	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
6	1.5×10^5	7.8×10^5	1.6×10^5	1.0×10^4	4.8×10^5	1.1×10^5
12	2.6×10^6	1.9×10^6	7.0×10^5	1.0×10^4	1.5×10^5	2.9×10^5
18	2.9×10^6	2.4×10^6	1.3×10^5	2.6×10^5	3.6×10^5	2.4×10^5
28	2.3×10^9	1.7×10^9	1.3×10^5	3.4×10^5	2.0×10^5	4.9×10^4

TABLE 6

Mean bacterial counts per gram of fat ground beef in package types 2, 4a, 6a, 8, and 15 and stored at 34–38° F. for 28 days. Run O.

(Mean count of 6 replicates)

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
	0	8.17×10^1	4.43×10^1	8.5×10^0	.....	8.58×10^0	6.60×10^0
2	7	1.25×10^5	1.18×10^5	6.17×10^2	.....	3.98×10^4	1.79×10^4
	14	1.83×10^6	1.09×10^6	1.55×10^5	2.95×10^5	1.01×10^5	1.80×10^5 *
	21	1.30×10^6	1.19×10^6	4.74×10^4	6.12×10^4	5.46×10^5	1.64×10^5
	28	1.60×10^6	1.92×10^5	5.73×10^4	1.37×10^5	1.12×10^5	1.74×10^5
	7	8.29×10^4	7.13×10^4	9.62×10^2	.....	3.45×10^4	2.68×10^4
4a	14	2.94×10^6	2.74×10^6	2.18×10^5	7.15×10^4	3.62×10^5	4.28×10^5
	21	5.85×10^6	5.04×10^6	2.49×10^5	3.89×10^5	4.50×10^5	6.94×10^5
	28	1.21×10^7	4.19×10^5	2.99×10^5	1.17×10^7	1.27×10^6	1.98×10^6
	7	3.14×10^5	2.89×10^5	2.43×10^4	.....	6.24×10^4	8.50×10^4
6a	14	1.78×10^6	1.55×10^6	1.51×10^5	8.36×10^4	3.98×10^5	1.90×10^4 *
	21	9.19×10^6	8.50×10^6	3.31×10^5	3.63×10^5	5.36×10^6	1.23×10^6
	28	9.04×10^6	1.00×10^5	6.03×10^5	7.42×10^6	1.42×10^6	1.39×10^6
	7	2.02×10^5	1.62×10^5	3.96×10^4	.....	9.73×10^4	7.79×10^4
8	14	2.65×10^7	2.47×10^7	1.40×10^6	6.90×10^5	2.32×10^6	5.70×10^6
	21	3.52×10^7	2.93×10^7	1.64×10^6	4.47×10^6	1.60×10^7	6.73×10^6
	28	2.72×10^7	4.65×10^6	7.15×10^5	2.26×10^7	8.58×10^6	3.71×10^6
	7	6.29×10^4	5.41×10^4	8.31×10^2	.....	1.97×10^4	1.12×10^4
15	14	2.04×10^5	1.72×10^5	1.33×10^4	2.38×10^4	3.98×10^5	1.90×10^4 *
	21	6.51×10^5	5.64×10^5	5.61×10^4	3.66×10^4	4.59×10^5	1.29×10^5
	28	1.06×10^6	1.70×10^5	3.21×10^4	9.02×10^5	1.16×10^5	9.69×10^4

* Mean count of 2 replicates.

TABLE 7

Mean bacterial counts per gram of fat ground beef in package type 2 held at room temperature for 20 hours before storage at 34–38° F. for 28 days. Run O.

(Mean count of 3 replicates)

Days of storage	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
7	1.93×10^6	1.25×10^6	4.81×10^4	1.04×10^6	6.25×10^5	1.71×10^5
14	2.90×10^6	2.64×10^6	2.41×10^5	1.03×10^5	1.99×10^5	1.18×10^5
21	1.52×10^6	2.79×10^5	7.13×10^4	1.25×10^6	2.91×10^5	5.81×10^4
28	1.46×10^6	6.60×10^4	4.97×10^4	1.43×10^6	1.22×10^5	1.98×10^4

TABLE 8

Mean bacterial counts per gram of fat ground lamb in package types 2 and 15 stored at 34–38° F. for 10 days. Run P.

(Mean count of 6 replicates)

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
	0	1.33×10^2	3.32×10^2	1.52×10^2	1.00×10^1	7.48×10^1	7.33×10^1
2	2	2.06×10^2	6.68×10^2	5.98×10^2	.....	8.55×10^1	7.38×10^1
	4	1.18×10^4	5.88×10^3	9.25×10^2	.....	3.31×10^2	3.91×10^2
	7	1.43×10^5	8.83×10^4	2.54×10^4	8.60×10^2	1.13×10^4	2.23×10^4
	10	3.60×10^6	2.52×10^6	1.11×10^6	8.55×10^3	2.49×10^5	3.60×10^5
	2	2.10×10^2	8.03×10^2	8.28×10^2	.....	8.20×10^1	6.83×10^1
15	4	1.59×10^4	7.19×10^3	7.88×10^2	.....	3.33×10^2	3.33×10^2
	7	1.06×10^5	2.16×10^4	8.18×10^4	3.45×10^2	1.14×10^3	5.61×10^3
	10	2.32×10^6	1.72×10^6	6.00×10^5	3.46×10^4	2.74×10^5	3.24×10^5

TABLE 9

Mean bacterial counts per gram of Achromobacter-Pseudomonas non-pigmented sp. inoculated fat ground lamb in package types 2 and 15, stored at 34–38° F. for 10 days.

(Mean count of 6 replicates)

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase	Oxidase
	0	4.26×10^5	2.98×10^5	8.33×10^3	1.25×10^5	6.31×10^4	1.22×10^5
2	2	1.20×10^8	1.02×10^6	7.42×10^4	4.53×10^4	3.54×10^5	2.19×10^5
	4	8.47×10^6	7.30×10^6	9.47×10^5	2.74×10^5	1.30×10^6	2.12×10^6
	7	7.91×10^8	7.11×10^6	7.18×10^5	4.38×10^5	1.09×10^6	1.79×10^6
	10	9.01×10^8	8.54×10^6	4.95×10^5	2.36×10^5	7.81×10^5	1.34×10^6
15	2	1.11×10^6	6.65×10^5	4.79×10^4	3.74×10^4	2.22×10^5	2.25×10^5
	4	$1.58 \times 10^{6*}$	$1.33 \times 10^{6*}$	$2.45 \times 10^{5*}$	$8.60 \times 10^{4*}$	$2.43 \times 10^{5*}$	$1.33 \times 10^{6*}$
	7	1.92×10^7	1.68×10^7	2.17×10^7	4.47×10^5	3.61×10^6	4.34×10^4
	10	1.45×10^7	1.22×10^7	2.24×10^6	2.14×10^5	2.89×10^6	4.68×10^6

* Mean of 5 counts.

TABLE 10

Mean bacterial counts per gram of uninoculated and non-pigmented Achromobacter-Pseudomonas type inoculated ground lamb in package type 2 held 20 hours before storage at 34–38° F. for 10 days.

(Mean count of 3 replicates)

Package type	Days	Total	Lipase	Oxidase
Uninoculated				
2	2	1.76×10^7	6.23×10^6	9.70×10^5
	4	3.54×10^7	3.60×10^6	2.33×10^6
	7	$6.37 \times 10^{8*}$	$7.00 \times 10^{8**}$	$1.23 \times 10^{5*}$
	10	1.48×10^7	2.71×10^6	4.50×10^5
Inoculated				
2	2	9.35×10^6	1.80×10^6	1.04×10^6
	4	2.84×10^7	1.04×10^7	8.72×10^6
	7	1.70×10^7	3.90×10^6	4.93×10^6
	10	$1.34 \times 10^{7*}$	6.03×10^6	$5.56 \times 10^{6*}$

* Mean of 2 counts. ** Mean of 1 count.

TABLE 11

Mean bacterial counts in fat ground lamb stored at 38–42° F. in package types 2, 6d, 15, and 15 n.v.* Run R.

(Mean of counts)

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* type	Lactobacilli	*Pseudomonas-Fluorescens* type
	0	3.05×10^2	4.68×10^1	5.75×10^1	.....
2	2	3.13×10^2	9.13×10^1	3.72×10^1	.....
	4	1.92×10^3	1.62×10^3	2.23×10^2	.....
	7	8.75×10^5	7.39×10^5	9.35×10^4	9.83×10^4
	10	1.08×10^7	9.02×10^6	1.42×10^6	2.62×10^6
	14	3.65×10^6	3.33×10^6	2.57×10^5	1.68×10^5
6d	2	3.04×10^2	9.44×10^1	4.62×10^1	.....
	4	1.16×10^4	9.28×10^3	1.32×10^3	.....
	7	4.41×10^6	2.50×10^6	1.44×10^5	9.59×10^4
	10	2.51×10^8	2.17×10^8	2.56×10^7	7.54×10^6
	14	7.21×10^8	6.46×10^8	3.16×10^7	3.47×10^7
15	2	4.43×10^2	1.34×10^2	5.72×10^1	.....
	4	7.40×10^2	5.17×10^2	6.86×10^1	.....
	7	4.34×10^4	4.22×10^4	6.95×10^2	1.46×10^4
	10	3.30×10^5	2.93×10^5	1.41×10^4	1.02×10^4
	14	2.47×10^6	2.24×10^6	2.34×10^5	7.82×10^4
15 n.v.*	2	4.38×10^2	1.37×10^2	5.38×10^1	.....
	4	4.59×10^2	2.99×10^2	8.05×10^2	.....
	7	1.41×10^6	1.46×10^6	8.04×10^4	1.04×10^5
	10	2.20×10^7	1.89×10^7	2.36×10^6	5.60×10^4
	14	1.28×10^7	1.17×10^7	4.79×10^5	7.47×10^5

* 15 n.v. –cellophane pliofilm laminate sealed without vacuum.

The mean per cent of the components of the total bacteria counts from three packaging materials at each examination time is shown in Table 14. The non-pigmented *Achromobacter-Pseudomonas* group comprised about 85% of the total counts at 7, 14, and 21 days of storage at 34–38° F. At 28 days, non-pigmented *Achromobacter-Pseudomonas* counts were only 15% of the total while the pigmented *Pseudomonas* species comprised a greater percentage. This shift was a common one in prepackaged meats.

TABLE 12

Mean bacterial counts in fat ground pork in packages 2, 6d, and 15 and stored for 14 days at 40–44° F. Run S.

(Mean of 6 replicates)

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* group
	0	1.03×10^2	1.03×10^2
2	2	8.77×10^1	8.77×10^1
	4	1.17×10^3	1.17×10^3
	7	3.21×10^5	3.21×10^5
	10	4.23×10^6	4.23×10^6
	14	1.94×10^7	1.94×10^7
Film 6d	2	9.91×10^1	9.91×10^1
	4	1.07×10^4	1.07×10^4
	7	4.18×10^6	4.18×10^6
	10	6.49×10^7	6.49×10^7
	14	2.74×10^8	2.74×10^8
Film 15	2	6.07×10^1	6.07×10^1
	4	9.60×10^2	9.60×10^2
	7	1.22×10^4	1.22×10^4
	10	5.11×10^5	5.11×10^5
	14	3.43×10^6	3.43×10^6

TABLE 13

Mean bacterial counts in fat ground pork inoculated with non-pigmented Achromobacter-Pseudomonas group, and stored at 40–44° F. in packages 2, 6d, and 15. Run S.

(Mean of 6 replicates)

500 Ml. Inoculum per 25 lbs. Meat
1 ml. Inoculum = 2.41×10^9 Cells

Package type	Days	Total	Non-pigmented *Achromobacter-Pseudomonas* group
	0	8.50×10^8	8.50×10^8
2	2	2.45×10^7	2.45×10^7
	4	3.15×10^7	3.15×10^7
	7	3.84×10^7	3.84×10^7
	10	2.83×10^7	2.83×10^7
	14	4.26×10^7	4.26×10^7
Film 6d	2	5.38×10^7	5.38×10^7
	4	4.41×10^8	4.41×10^8
	7	5.67×10^8	5.67×10^8
	10	7.35×10^8	7.35×10^8
	14		
Film 15	2	5.38×10^6	5.38×10^6
	4	1.60×10^7	1.60×10^7
	7	1.30×10^7	1.30×10^7
	10	1.74×10^7	1.74×10^7
	14	2.21×10^7	2.21×10^7

At 40–44° F. the pigmented *Pseudomonas* group was dominant early in the storage period.

Lactobacillus species were also important components of the bacterial flora of prepackaged meats (Tables 3 through 11 and Figures 1 through 4). Counts of lactobacilli increased throughout the storage period, but usually were lower in number than those of the non-pigmented *Achromobacter-Pseudomonas* group. Table 14 shows that lactobacilli comprised a little more than 5% of the total count at each examination period, irrespective of the packaging conditions. The growth of the non-pigmented *Achromobacter-Pseudomonas* group apparently produced coonditions favorable to the lactobacilli.

Organisms of the *Pseudomonas-fluorescens* type, between the 21st and 28th days of storage at 34–38° F. and between the 10th and 14th days of storage at 40–44° F. constituted approximately 85% of the total population.

TABLE 14

Mean percent of components of the total bacterial counts during storage. Run O. (Fat ground beef stored at 34–38° F.)

Time (Days)	Non-pigmented *Achromobacter-Pseudomonas* group	Lactobacilli	*Pseudomonas-Fluorescens* type	Lipase producers	Oxidase producers
0	55.1	9.75	0.00	11.3	8.51
Can (2)					
7	90.4	8.77	0.00	30.50	16.70
14	77.7	11.00	13.90	4.55	4.81
21	91.3	5.25	4.36	40.40	13.10
28	10.8	3.87	87.40	8.99	16.70
MSAT80 Cellophane-coated side in. (6a)					
7	91.9	5.96	0.00	25.9	27.90
14	86.4	8.58	5.86	11.8	8.73
21	89.9	5.54	5.46	51.6	14.70
28	10.6	5.46	87.00	10.4	9.76
Cellophane Pliofilm Laminate (15)					
7	88.6	10.60	0.668	25.20	28.7
14	93.2	5.67	2.88	9.55	42.9
21	83.7	10.70	6.42	69.60	21.3
28	22.8	3.87	76.10	11.00	14.4

Relationship between the data of the selective media tests and the classifications results

Various selective media * were used concurrently with the veal infusion agar to determine whether or not population shifts could be detected by the differences in the cultural activities of the bacterial flora. Only 3 selective media gave promising results; therefore, they were used until the classification studies were completed. These were Jensen's lard agar for oxidase formers, Turner's nile blue sulfate lard agar for lipase producers, and gelatin peptone agar.

Tables 15 through 19 show the changes in the counts of the gelatin liquefiers, oxidase formers, and the lipase producers with respect to the total counts of prepackaged meats at each examination time. The total counts from the selective media were always lower than those on the veal infusion agar. Possibly the medium was slightly inhibitory toward bacteria cells. Increases in the total counts during the storage period are usually followed by proportionate increases in the selective media counts. Correlation coefficients were calculated to ascertain the significance of this relationship.

TABLE 15

Correlation* between total bacterial counts and oxidase counts (Can samples—fat ground meat stored at 34–38° F.)

Run	Kind of meat	Sum of log total count	Sum of log oxidase count	"r"	P. E. of "r"
G	Beef	87.05	73.72	0.995	0.0029
H	Beef	66.93	55.08	0.935	0.0379
I	Lamb	90.68	76.67	0.963	0.0194
J	Beef	138.28	112.19	0.635	0.1406
M	Pork	164.09	125.92	0.617	0.1189
N	Lamb	162.89	127.93	0.963	0.0013

* The method for the calculation of the correlation between two variables is that of Snedecor *(1)*. The correlation coefficient (r) is highly significant in all the results tabulated.

TABLE 16

Correlation between total bacterial counts and oxidase counts. (Cellophane pliofilm laminate (15)—fat ground meat stored at 34–38° F.)

Run	Kind of meat	Sum of log total count	Sum of log oxidase count	*"r"	P. E. of "r"
G	Beef	92.27	78.37	0.977	0.0126
H	Beef	56.30	47.95	0.905	0.0549
I	Lamb	86.03	67.95	0.882	0.0594
J	Beef	108.39	87.09	0.927	0.0376
M	Pork	174.54	126.14	0.548	0.1299
N	Lamb	155.08	115.46	0.523	0.1349

* The correlation coefficient is significant in all the results tabulated.

TABLE 17

Correlation between total bacterial counts and oxidase counts (Fat ground meat stored at 34–38° F.)

Run	Kind of meat	Sum of log total count	Sum of log oxidase count	*"r"	P. E. of "r"
MSAT86 Cellophane (Package Type 6)					
H	Beef	72.45	61.46	0.949	0.0300
Cellulose Acetate Pliofilm Laminate (Package Type 15)					
I	Lamb	91.18	79.15	0.982	0.0095
J	Beef	110.93	91.33	0.881	0.0600

* The correlation coefficient is highly significant in all the results tabulated.

Correlation between the total bacteria counts and the oxidase counts

A significant correlation indicated that the rate of change of the total bacterial count was similar to the oxidase rate of change (Table 15). In Runs G, H, I, and M, the oxidase counts of the meat packaged in the can (2) increased at the same rate as the respective total bacteria counts. A lower degree of significance was found in Runs J and N.

* Composition of the media used in the plating procedure per liter:

I. *Veal Infusion Agar*
23 g. Difco Veal Infusion
15 g. Difco Agar
2.5 g. Sodium Chloride C. P.

II. *Jensen's Lard Agar (Modified)*
23 g. Difco Nutrient Agar
10 g. Gum Tragacanth
133 ml. Melted Lard
Food plates with 0.1% Kodak tetramethyl-p-phenylenediamine dihydrochloride

III. *Turner's Nile Blue Sulfate Lard Agar (Modified)*
23 g. Difco Nutrient Agar
10 g. Eimer and Amend Gum Tragacanth
1.0 g. Nile Blue Sulfate previously dissolved in water and filtered
133 ml. Melted Lard

IV. *Gelatin-peptone Agar*
4 g. Difco Peptone
3 g. Difco Gelatin
15 g. Difco Agar
Flood plates with 0.1% acidified mercuric chloride.

TABLE 18

Correlation between total bacterial counts and lipase counts (Fat ground meat stored at 34–38° F.)

Run	Kind of meat	Sum of log total count	Sum of log oxidase count	*"r"	P. E. of "r"
Can (Package Type 2)					
J	Beef	138.28	108.18	0.450	0.1880
M	Pork	164.09	132.66	0.730	0.0883
N	Lamb	162.89	127.99	0.951	0.0175
O	Beef	150.90	122.56	0.952	0.0171
P	Lamb	126.41	84.83	0.907	0.0329
MSAT80 Cellophane (Package Type 6a)					
O	Beef	159.59	133.45	0.969	0.0211
Cellophane Pliofilm Laminate (Package Type 15)					
O	Beef	139.19	116.98	0.967	0.0118

* The correlation coefficient is highly significant in all the results tabulated.

TABLE 19

Correlation between total bacterial countst and gelatin liquefiers (Can samples—fat ground meat stored at 34–38° F.)

Run	Kind of meat	Sum of log total count	Sum of log oxidase count	*"r"	P. E. of "r"
G	Beef	92.27	73.71	0.861	0.0747
I	Lamb	86.03	77.46	0.958	0.0022

* The correlation coefficient is highly significant in all the results tabulated.

The data from other packaging materials, shown in Tables 16 and 17, further corroborated the hypothesis that the rate of change in the total bacterial counts was coordinated with the rate of change in the oxidase counts.

Since the oxidase counts increased with time, the organisms responsible for this activity must be able to grow at 34–38° F. Members of the *Achromobacter-Pseudomonas* group were probably responsible for this activity.

Correlation between the total bacteria counts and the lipase counts.

Table 18 shows that there was a positive linear correlation between the total bacteria counts and the lipase producer counts, throughout the storage period. Cultural tests indicated that lipase counts were proportional to the counts of the *Achromobacter-Pseudomonas* group, thus indicating that these organisms produced lipase.

Correlation between the total bacteria counts and the gelatin liquefiers

A significant linear relationship was found between the rate of change in the total bacteria counts and the rate of change of the gelatin liquefier counts with time (Table 19). The *Achromobacter-Pseudomonas* group was responsible for the positive results of liquefaction of the gelatin-peptone agar culture medium.

SUMMARY

Bacterial flora of prepackaged meats. Two groups of bacteria predominated the bacterial flora of fresh meats during a four-week storage period at 34–38° F.

The morphological growth characteristics of *Achromobacter-Pseudomonas* and lactobacillus colonies on the surface and in the sub-surface of a culture medium were sufficiently distinct to make possible the differentiation of the genera from each other in a mixed culture by inspection. During the first two weeks lactobacilli and non-pigmented organisms of the *Achromobacter-Pseudomonas* type predominated, while in the later part of the storage period those of the *Pseudomonas-fluorescens* type became dominant.

Non-pigmented *Achromobacter-Pseudomonas* type organisms constituted about 85% of the population during the first two weeks of storage at 34–38° F. and during the first week at 40–44° F. During the entire examination period lactobacilli accounted for about 5% of the total population. Bacteria of the *Pseudomonas-fluorescens* type, constituted approximately 80% of the total count in the latter part of the storage period. In the early part of the storage period they seldom exceeded 5% of the population. The packaging materials did not selectively affect the rates of growth of the predominant genera.

Use of selective media to determine the bacterial population shifts was unsatisfactory since the flora itself was selective in nature.

A significant mathematical correlation was found between the total bacteria counts and the counts obtained from the selective media used. Thus, the rate of increase in the number of bacterial cells, which produced oxidase or lipase, or liquefied gelatin, was coordinate with the rate of increase in the total bacteria counts.

LITERATURE CITED

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Inhibition of Pectinolytic and Cellulolytic Enzymes in Cucumber Fermentations by Scuppernong Grape Leaves [a]

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THE ANNUAL CROP of pickling cucumbers in this country from 1954 to 1956 has been reported *(4)* at 12 to 14 million bushels with a farm value of more than 21 million dollars to an estimated 50,000 growers. The pickle industry itself has more than doubled in the past 20 years and it is becoming increasingly important that the processing of this crop, worth over 165 million dollars at the manufacturers level, be eventually placed under controlled conditions with a minimum of loss due to spoilage.

One of the spoilage problems that has confronted the industry for many years is that of enzymatic softening of the cucumbers during brine fermentation and storage. It has been estimated *(8)* that this type of spoilage represents an annual loss of about one million dollars, particularly where the salt-stock cucumbers have deteriorated in firmness to the point where they have to be discarded or at best used in low-quality, low-priced products.

The nature of salt-stock softening under commercial conditions in the South and Southwest has received considerable investigation *(2, 3, 6, 7, 8)*. Results demonstrate that the softening is the result of pectinolytic and cellulolytic enzyme activity in the brine and that these enzyme systems are introduced into the curing vats chiefly by way of the partially dried cucumber flowers that remain attached to the cucumber fruit. In laboratory tests *(2)* purified polygalacturonase has been demonstrated to soften salt-stock; however, the exact role of the cellulolytic enzyme in the brines has not been clearly established.

Microbial studies strongly indicate that the enzyme activity is chiefly the result of growth by higher fungi (molds) in the cucumber flowers prior to their reaching the brining station. The principal species of molds on the growing cucumber plant have been isolated and studied as to their biochemical properties *(7)*. As a group, most of them are a potent source of various hydrolytic enzymes which cause a break-down of pectic and cellulosic substances.

Several approaches directed toward eliminating or significantly reducing the concentration of softening enzymes in commercial cucumber brines have been suggested *(3, 8)*. These are: (a) mechanical removal of flowers from the cucumbers; (b) development of new cucumber varieties with a minimum of retained flowers; (c) development of draining procedures to reduce enzyme content of brines before it can affect cucumber firmness; and, (d) inactivation of the enzymes in the brine with specific, non-toxic inhibitors. The draining procedure has received rather thorough investigation *(8)* and has been widely accepted in southern brining areas.

The use of specific, naturally occurring, non-toxic inhibitors is the approach considered in the present study to control enzymatic softening of brined cucumbers.

In 1953, Weurman *(17)* reported the first naturally occurring thermolabile inhibitor for pectinase in several varieties of pears; also, he was able to precipitate the active substance from the pear sap with acetone. A number of naturally occurring inhibitors have been reported for other hydrolytic enzymes, such as the amylase inhibitors from wheat and Leoti sorghum *(11, 15, 16)*, and the trypsin inhibitors from soybean, lima bean, colostrum, and pancreas *(12)*. Bell and Etchells *(1)* have recently found that pectinolytic and celluloytic enzymes are inhibited by a water soluble fraction of grape leaves. Among the 6 varieties of grapes studied, the leaves of a native American variety—namely, Scuppernong of the Muscadine group *(Vitus rotundifolia Michx.)*—gave the highest inhibitor concentration per gram of leaves.

Use of grape leaves in making fermented dill pickles for home use was suggested by LeFevre *(13)* in 1927. He states: "It is a good plan to place over the top a layer of grape leaves. In fact, it would be well to place these at both the bottom and top. They make a very suitable covering and have a greening effect on the pickles." The use of grape leaves in cucumber pickles has been handed down from one generation to another in home pickling procedures and may be found in very old recipe books. However, the true value of grape leaves has not been demonstrated in the scientific literature.

MATERIALS AND METHODS

In the present work, Scuppernong grape leaves were used as

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[b] One of the laboratories of Southern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

inhibitor source for the softening enzymes; this inhibitor is referred to throughout as GLI. The effect of this material on small-scale, experimental cucumber fermentations was followed for: (a) enzyme activity; (b) brine acidity and pH; (c) microbial activity during fermentation as indicated by optical density of the brine; and, (d) firmness and general acceptability of the cucumbers after approximately 90 days of fermentation and curing in brine.

The enzyme systems measured in this paper were (a) polygalacturonase (syn. pectinase), which hydrolyzes the glycosidic bonds of pectic acid with simultaneous loss in viscosity and increase in reducing groups; and (b) cellulase (Cx) which hydrolyzes the glucosidic bonds of a soluble cellulose derivative (sodium carboxymethyl-cellulose) with loss in viscosity and an increase in reducing groups. These two systems will be referred to as "pectinolytic activity" and "cellulolytic activity."

Grape leaf inhibitor (GLI). Approximately 20 pounds of Scuppernong leaves were picked at the Coastal Plain Experiment Station, Willard, North Carolina. The leaves were cooled to approximately 5° C. within 2 hours after picking, and held at this temperature overnight. After washing twice in cool tap water, they were air dried to approximately their original weight. Two lots of 500 g. each were placed in an air flow oven at 45–48° C. for 40 hours, then ground in a Wiley mill using 0.5 mm. sieve and stored as a powder at room temperature. The remaining leaves were placed in large polyethylene freezer-bags, sealed, and stored at −10° C. Later, the frozen leaves were shipped with dry-ice to the pickle plant just prior to use in the cucumber-fermentation studies.

Source of cucumbers and cucumber flowers. Eighty bushels of No. 1 size (1 in. diameter) Model variety cucumbers, representing 20 bushels from each of four receiving stations, were de-flowered by hand. Additional flowers were obtained from the grading machines at the same four receiving stations. Flowers were separated from sand, grit, and other extraneous material by the use of hardware cloth wire screens, followed by picking over by hand. In all, about 15 pounds of flowers were obtained and in order to assure uniform enzyme concentration these were thoroughly mixed by tumbling in large boxes.

Brining procedure and sampling. Well coopered, clean 45-gallon barrels were used as containers for the brining experiments. The containers were well marked as to treatment and 215 lbs. of de-flowered cucumbers, representing a composite of the stock from the 4 receiving stations, were placed in each.

In the treatments employed (Table 1), flowers and/or GLI were added according to the design of the experiment. The GLI was added as a crude, brine-extract at the time the cucumbers were covered with brine. The extract was prepared by first chopping 2 ounces of grape leaves with a cleaver on a cutting board and then blending the minced leaves in 1 pint of 25° salometer brine (6.6% salt by wt.) with a Waring blender.[c] In this manner, the 7 lbs. of fresh leaves were prepared in 2 hours using 2 blenders.

Flowers were equally distributed as the cucumbers were added to the barrels. Next, each barrel was fitted with a "false" wooden head which covered the cucumbers and then about 20 gallons of 25° sal. brine were added. This amount brought the brine level to about 2 inches above the head and about the same distance from the top of the barrel. Twelve pounds of dry salt were added on the head of each barrel and the following day the brines tested 25° sal. (± 2°). This concentration was increased 5° sal. per week to a holding strength of 60° sal.

Barrels were stored under sheltered conditions and received constant attention throughout the 90-day curing period to preclude active surface yeast development. Brine temperatures during the active fermentation period were in the range of 28 to 30° C.

Barrels were sampled with the equipment described by Etchells and Jones *(9)* at the following fermentation periods: after 0, ¼, ½, 1, 2, 3, 5, 10 and 30 days. Brine samples were collected in 3-ounce bottles supplied with tin-lined caps and 5 drops of toluene were added to each sample as a preservative.

[c] Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentionel.

TABLE 1

Treatments employed in the small-scale cucumber fermentations with added cucumber flowers and grape leaf inhibitor (GLI)

Treatment[1]	Cucumber flowers added per barrel	GLI added per barrel
	pounds	*pounds*
A	0	0
B	1.25	0
C	1.25	0.25
D	1.25	0.75
E	1.25	1.25
F	0	1.25

[1] Each treatment consisted of duplicate 215 pound lots of no. 1 size Model variety cucumbers brined in 45-gallon barrels. The basic brining treatment, 25° sal. (6.6% salt) raised 5° sal. (1.3%) per week to a holding strength of 60° sal. (15.8%), represented commercial practice at the plant where the experimental work was done.

All samples were stored at about 5° C. until they were shipped by air to the laboratory for analysis. The experimental brining work was done during the 1956 season at a commercial pickling plant located in northeastern Texas.

Commercial pectinase experiment. An exploratory experiment was conducted later on during the season in which a commercial pectinase,[c, d] rather than the cucumber flowers, was used as the source of the enzyme. In this experiment there were 4 treatments in duplicate 45 gal. barrels; these were as follows: Treatment N, control, without enzyme or GLI; Treatment O, 8 g. of pectinase only; Treatment P, 8 g. of pectinase plus 0.25 lb. GLI; and, Treatment Q, 8 g. of pectinase plus 0.75 lb. GLI. The salting procedure, brine sampling and other details were the same as for the first experiment.

Brine analysis. Pectinolytic and cellulolytic enzyme activities of the brine samples were measured by the viscometric method described by Bell *et al. (3)*. In this procedure, 100 units of pectinolytic activity equal 50% loss in viscosity in 20 hours of 1.0% sodium polypectate solution in a citrate buffer at pH 5.0, at 30° C. The cellulolytic activity units are equal to same conditions as above except 1.0% sodium carboxymethylcellulose was used as the substrate. Total acidity was measured using 10 ml. aliquots of the brine samples and titrating with 0.111 N NaOH, using phenolphthalein as the indicator. The acidity is expressed as grams of lactic acid per 100 ml. sample. Brine pH was measured with the Beckman[c] pH meter, Model H-2. Optical density of the brines was taken with Lumetron[c] colorimeter using a 650 mμ filter and 18 mm. diameter test tubes.

The cured salt-stock was judged by personnel of the pickle company for color and acceptability for commercial use; this procedure was essentially that described by Jones *et al. (10)* except the quality categories were increased from 3 *(not acceptable, barely acceptable,* and *acceptable)* to 5 *(not acceptable, poor, fair, good,* and *excellent)*. Later, the stock was manufactured into sweet pickles and processed dill pickles. These were judged for *appearance, flavor, texture,* and *overall acceptability*. Each of these 4 pickle characteristics was scored as to one of the following ratings: *not acceptable, poor, fair, good,* and *excellent*. The U.S.D.A. Fruit Pressure Tester *(14)* was used for determining the firmness of the salt-stock *(3)*.

RESULTS

Effect of GLI on pectinolytic and cellulolytic enzymes. Pectinolytic and cellulolytic activities of the brine samples at the maximum level (24 hr. period) and the percentage of enzyme inactivation by GLI are presented in Table 2. Maximum enzyme activities in all brines were found 24 hours after the cucumbers were covered with brine. These results are in general agreement with studies by Etchells *et al. (7)* on the maximum enzyme level reached in flower-added brines after a similar time interval. However, the pectinolytic activity of the cucumber flowers used in the present study was much lower than those in the above report. Due to the conditions of the experiment, it was not possible to measure enzyme activity of the cucumber flowers at the plant prior to starting the tests

[d] Pectinase 46 AP (No. 32) was supplied by Rohm and Haas Company, Philadelphia, Pennsylvania.

and thus forecast the approximate activity of softening enzymes in the brines. It is important to mention that the enzyme activity of cucumber flowers from No. 1 size stock may vary greatly not only between seasons but also for given periods within the same season.

The 3 control treatments, A (cucumbers only), B (cucumbers plus flowers), and F (cucumbers plus GLI) reached levels of pectinolytic activity in the brine of 13, 35, and 2 units respectively. In comparing treatments A and B, the enzyme activity contributed by the cucumbers represented about one-third of the total enzyme activity found; in Treatment F (cucumbers plus GLI) the activity was 2 units as compared with 13 units for A (cucumbers only). This represented an 85% reduction in pectinolytic activity caused by the addition of GLI. In treatments C, D, and E, with 0.25, 0.75, and 1.25 pounds of GLI added per barrel respectively, the pectinolytic enzyme activity levels were reduced from 35 units (enzyme control) to 20, 10 and 6 units or 43, 71 and 83%.

The results for inhibition of cellulolytic enzyme activity in the different treatments followed very closely those discussed for pectinolytic activity (Table 2). The total cellulolytic enzyme concentration provided by the flowers and cucumbers, as shown by the maximum level at the 24-hour period, was in the same range (200 units) as reported in earlier studies *(7)*. In the 3 control treatments, the cucumbers alone contributed 22 cellulolytic units (A), the cucumbers plus flowers 240 units (B), and, the cucumbers plus GLI 5 units (F). The cucumbers added less than 10% of the total enzyme activity to the cucumber flower treatments, and the GLI reduced this activity about fourfold. In treatments C, D and E, with 0.25, 0.75, and 1.25 pounds of GLI added per barrel respectively, the cellulolytic enzyme levels were reduced from 240 units (enzyme control) to 172, 118, and 63 units. The percentages of inactivation caused by these three levels of inhibitor were 28, 51, and 74% respectively.

Figure 1 presents the pectinolytic and cellulolytic activity levels during the first 5 days of fermentation for the 1.25 pound GLI treatment (E) as compared to the enzyme control without inhibitor (B). The reduction of activity of both enzyme systems by GLI was very pronounced in these cucumber fermentations.

Firmness of salt-stock. An important objective of these inhibitor treatments was to correlate, if possible, the reduction of softening enzyme activity with improved firmness of cured brine-stock cucumbers. In considering treatments A (no flowers or inhibitor added) and B (flowers only added), the addition of flowers to the fermentation reduced the firmness as measured by the U.S.D.A. pressure tester, from 19 to 13 pounds, a loss of 32%. When the three increasing levels of GLI (C, D, E) were added to the cucumbers plus flower treatment, the per cent loss in firmness decreased (26, 11, and 0%). The highest inhibitor level (E, 1.25 pounds of GLI), gave salt-stock testing 19 pounds; this was equal in firmness to the control treatment (A) with no flowers or inhibitor added.

The influence of inhibitor concentration on relative enzyme (pectinolytic and cellulolytic) activities in the brines at the 24-hour observation period in relation to firmness of the cured salt-stock cucumbers is presented in Figure 2. As the inhibitor

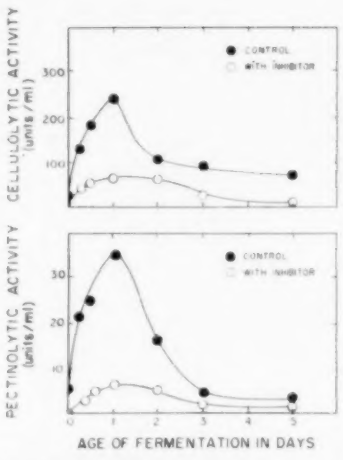

Figure 1. Effect of grape leaf inhibitor (1.25 lbs./bbl.) on pectinolytic and cellulolytic enzyme activity in cucumber brines.

concentration was increased, the relative pectinolytic and cellulolytic enzyme activities decreased and the firmness of the cured salt-stock increased. The firmness of the stock was found to be in direct relationship to inhibitor concentration and inversely related to the activity of the two enzyme systems.

Salt-stock and pickle quality. A panel of two experienced salt-stock judges evaluated coded, 50-pound lots of cured material from the experimental fermentations. Treatments receiving increasing levels of GLI (C, D, and E) were rated *good* to *excellent* as to *acceptability for commercial use*. This was the same rating given salt-stock from Control Treatments A and B. The 32% loss in firmness of Treatment B stock was not sufficient to influence the judges' ratings; experience has shown that an approximate loss of 50% is required to be readily detected by hand, even by the most experienced judges. The judges down-rated material from Treatment F—representing GLI only—to the *fair* category because it was considered to have a darker color than desired for salt-stock purposes. However, no marked color differences were obtained when salt-stock from all GLI treatments was de-salted and manufactured into processed dill pickles and sweet pickles. Furthermore, these pickles received a quality rating of *good* for *overall acceptability* by a panel of 10 judges representing three different pickle companies.

Total acidity, pH, and optical density of the brine samples. The 6 treatments were brined essentially the same except for the possible effect of added GLI and/or cucumber flowers in certain lots. The total acidity and pH of the brine samples from the 1st to the 10th day are given in Table 3. The rate of brine acid development in all treatments was very consistent and typical of a 25° salometer fermentation. A very rapid rise

TABLE 2

Pectinolytic and cellulolytic enzyme activity of the brines 24 hours after brining and percentage inactivation of the enzymes by grape leaf inhibitor (GLI)

Treatment[1]	Pectinolytic enzyme		Cellulolytic enzyme	
	Activity units/ml.[2]	Inactivation by GLI	Activity units/ml.[2]	Inactivation by GLI
		%		%
A: Control, no flowers, no GLI	13	0	22	0
B: Control, flowers, no GLI	35	0	240	0
C: Flowers + 0.25 lb. GLI	20	43	172	28
D: Flowers + 0.75 lb. GLI	10	71	118	51
E: Flowers + 1.25 lbs. GLI	6	83	63	74
F: Control, no flowers + 1.25 lbs. GLI	2	85	5	77

[1] 1.25 lbs. cucumber flowers added per bbl. as indicated; GLI = grape leaf inhibitor added as idicated.
[2] Average of duplicate lots.
NB: The average firmness (in pounds) of the cured salt-stock from the various treatments was as follows: A, 19 lbs.; B, 13; C, 14; D, 17; E, 19; and F, 18.

in acid development occurred within 3 days of fermentation and maximum acidities from 0.51 to 0.62% were reached after 10 days. The increase in brine acidity was accompanied by a corresponding decrease in brine pH during the 10-day period. The pH values dropped from above 5 on the 1st day to 3.6 for all treatments on the 3rd day. The optical density (O.D. × 10) of the brine samples reached a peak between the 3rd to 5th day with values in the range of 3.0 to 4.0; this indicated an early, vigorous lactic acid fermentation.

Based on the above results, the addition of grape leaves appeared to exert no noticeable inhibiting effect on the lactic acid bacteria responsible for the acid fermentation in the various experimental treatments.

Commercial pectinase experiment. The pectinolytic enzyme

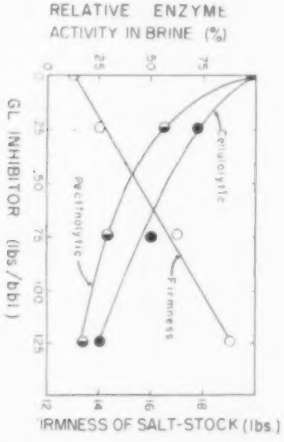

Figure 2. Effect of grape leaf inhibitor on maximum pectinolytic and cellulolytic enzyme activity in cucumber brines as related to firmness of cured salt-stock.

activity of the commercial material was extremely high and the addition of 8 grams per barrel was far in excess of that found in commercial brines with soft salt-stock. The use of 8 g. of enzyme without inhibitor (Treatment O) resulted in a maximum pectinolytic enzyme activity of 57,000 units at 24 hours; 100 units in the brnie is usually sufficient to reduce salt-stock firmness 50%. The addition of 0.25 pound of GLI per barrel (Treatment P) reduced this activity 25% and 0.75 pound of GLI per barrel (Treatment Q) reduced the activity about 50%. With the exception of control Treatment N (without pectinase and without GLI), the salt-stock from the pectinase treatments was extremely soft.

Dried GLI experiment. Laboratory tests on the dried preparation made from grape leaves demonstrated that it was only about one-third as effective as an equivalent amount of frozen and thawed material in inhibiting pectinolytic enzyme activity. A limited brining study—using one level of dried GLI (equivalent to 1.25 lbs. of fresh leaves)—further revealed an even more drastic loss in ability to inhibit cellulolytic activity. In addition, salt-stock cucumbers from the above test showed no improvement in firmness over material from flower-added controls.

DISCUSSION

The inhibition of pectinolytic and cellulolytic activity by the crude brine extract of Scuppernong grape leaves offers a most promising lead to the ultimate chemical control of softening activity in commercial cucumber brines. It is obvious that the addition of such large quantities of grape leaves to commercial vats of cucumbers would not be practical. However, with further characterization and isolation of the active inhibitor substance, coupled with further investigations in the screening of plant materials to obtain higher concentrations of the active ingredient, this pectinolytic and cellulolytic inhibitor would be most valuable to the pickle industry and possibly other industries where the control of the degradation of pectin and cellulose is desirable.

The potential use of such an inhibitor might well extend into the fields of fungicides and insecticides. The relative freedom from disease and insect attack enjoyed by leaves of the Scuppernong *(5)* may well be the result of this naturally occurring enzyme inhibitor. Such a compound could be envisioned as inhibiting certain enzyme systems (i.e. cellulase) of the attacking fungi and insects and thereby limiting utilization of this plant material as a source of nutrients.

Information bearing on the purification, characterization, and chemical properties of the inhibitor(s) in grape leaves will be reported in another paper.

SUMMARY

Pectinolytic and cellulolytic enzymes in cucumber flowers, when added to small-scale fermentations, were effectively reduced in activity by the use of a crude extract of Scuppernong grape leaves *(Vitus rotundifolia)*. There was no apparent influence of the grape leaf inhibitor (GLI) on the character of the acid fermentation as measured by total acidity, pH, and optical density of the brine. The reduction in activities of the two enzyme systems was directly related to the inhibitor concentration used; higher levels of inhibitor resulted in an increase in firmness of the fermented cucumbers (salt-stock). In general the judges at the pickle plant rated the salt-stock *good* to *excellent* as

TABLE 3

Total acidity and pH of the brines from the experimental treatments during 10 days of fermentation

Treatment[1]	Fermentation in days					Fermentation in days				
	1	2	3	5	10	1	2	3	5	10
	Total acidity as lactic[2]					Brine pH[2]				
	%	%	%	%	%					
A: Control, no flowers, no GLI	.08	.32	.37	.39	.54	6.2	3.7	3.6	3.6	3.5
B: Control, flowers, no GLI	.09	.43	.51	.51	.45	5.8	3.7	3.6	3.6	3.5
C: Flowers + 0.25 lb. GLI	.08	.28	.50	.52	.55	5.6	4.0	3.6	3.6	3.5
D: Flowers + 0.75 lb. GLI	.09	.27	.51	.56	.62	5.6	4.5	3.6	3.5	3.4
E: Flowers + 1.25 lbs. GLI	.08	.25	.54	.57	.55	5.4	4.4	3.6	3.5	3.4
F: Control, no flowers + 1.25 lbs. GLI	.06	.19	.50	.50	.48	5.9	4.7	3.6	3.6	3.5

[1] 1.25 lbs. cucumber flowers added per bbl. as indicated; GLI = grape leaf inhibitor added as indicated.
[2] Average of the duplicate lots.

to *acceptability* for *commercial use* and the only comment was "darker color" for material from some of the GLI treatments. However, when salt-stock was finished into processed dill pickles and sweet pickle products, there was no appreciable difference in color of the GLI treated stock over the control. Furthermore, the pickles made from all GLI lots received a quality rating of *good* for *overall acceptability*.

Acknowledgments

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Theoretical Considerations in Food Processing by Gamma Radiations

II. A Simplified Mathematical Procedure for Evaluating Gamma Radiation Processes Inside Hollow Cylinder Sources[a]

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(Manuscript received August 15, 1957)

THE SUGGESTED USE of gamma radiation for food processing presents the problem of determining the least exposure time to yield a safe product from a microbiological standpoint. The Vitro Corporation, in 1952, presented the basic equations for evaluating dose rate at the center of a hollow cylinder source. However, they neglected to consider some of the fundamental factors involved in determining a safe exposure time *(5)*, such as initial numbers of organisms, shape and size of the container, and radioresistance of the most dangerous resistant organism associated with the product.

In 1953, Henley and Gaden *(3)* noted the analogy between thermal process evaluation and radiation process evaluation. Their method involved the experimental determination of the dose rate inside the container an dthe use of previously constructed nomographs. They attempted to integrate the lethal effects of radiation through the container, and although they realized that various regions of the container were subject to different dose rates, they did not take into account the fact that different initial numbers of organisms were also exposed to the various dose rates.

Charm, Goldblith, and Proctor *(1)* have developed a procedure for evaluating radiation processes by graphical determination of the dose rate distribution throughout the container and coordination of the results of such a determination with recent concepts of thermal processing. A simpler mathematical procedure has now been devised for the evaluation of gamma radiation processes inside a hollow cylinder source.

DEVELOPMENT OF SIMPLIFIED EQUATION

It had been observed that the plot of average dose rate in a hollow cylinder (Figure 3) referred to radius appeared to approximate a parabola having the equation:

$$C^2 = M\,(\overline{D}_{(C)} - \overline{D}_{(A)} \qquad (1)$$

where:

C = distance along the radius, cm.

M = Constant

$\overline{D}_{(C)}$ = average dose rate at C units from the center line, rep/ sec.

$\overline{D}_{(A)}$ = average dose rate at the center line, rep/sec.

The shapes of several dose distribution curves reported in the literature were checked and found to be parabolic except at the outer regions. However, the outer regions are not critical in determining the numbers of survivors, because of the severity of the dose received in the outer regions compared with the severity of doses at points nearer the center. The parabolic equation is, therefore, satisfactory for expressing the dose rate in these cases.

It was necessary to determine what effect the dimensions of the source and various characteristics of the fission product have on the shape of the parabolic dose rate distribution. The average dose rate for a distance of C = 0.68b (where b = radius of the source) was calculated for sources of various radii and various ratios of length to radius. The method described by Charm, Goldblith and Proctor *(1)* was employed for this purpose. The following equations were solved graphically, to determine the average dose rate $\overline{D}_{(C)}$ where C = 0.686b

$$D_{(P)} = \frac{2KC_Ab}{4\pi} \int_0^L \int_0^\Pi \frac{e^{-\mu_P \sqrt{l^2 + C^2 + b^2 - 2Cb\cos(\theta)}}}{l^2 + C^2 + b^2 - 2Cb\cos(\theta)}\, d\theta dl \qquad (2)$$

$$\overline{D}_{(C)} = \frac{1}{L} \int_0^L D_{(p)}\, dl \qquad (3)$$

where:

$D_{(p)}$ = Dose rate received at a point a distance "C" from the center line of the source and a distance "l" from the entrance of the source, rep/sec.

L = Length of source, cm.

K = Conversion factor to convert average energy *E* of a fission product source from curies/to rep/sec.

C_A = Strength of source per unit area (curies/ sq. cm.)

b = Radius of source cm.

μ_P = Absorption coefficient of absorber

[a] Contribution No. 337 from the Department of Food Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

C = Distances measured along radius from center line, cm.

l = Distances measured along center-line from entrance, cm.

θ = Central angle in plane passing through cross section of cylinder (Fig. 4).

$\overline{D}_{(C)}$ = Average dose rate at distance "C" from center line of source, rep/sec.

Results were plotted as a series of curves (Figure 1, Table 1). Knowledge of the corresponding average dose rates along the center line of the source for the various dimensions of the source was also required for the determination of the parabolic dose rate distribution. The average dose rate at the center of the source was calculated from the series of curves determined by the Vitro Corporation *(5)* (See Figure 2). From these calculations it was found that:

$$\frac{\overline{D}(0.686b)}{C_AK} - \frac{\overline{D}(center)}{C_AK} = 0.18 \qquad (4)$$

See appendix, Tables 1, 2, 3 and Figures 1 and 2.

This makes it possible to express M, which is related to the *latus rectum* of a parabola, in terms of b, C_A, and K.

Since $C^2 = M\,(\overline{D}_C - \overline{D}_A)$

and $\overline{D}_{(0.686b)} - \overline{D}_{(A)} = 0.18\ C_AK$

when $C = 0.686b$

Then $(0.686b)^2 = 0.18\ C_AKM \qquad (5)$

or $\dfrac{2.62b^2}{C_AK} = M$

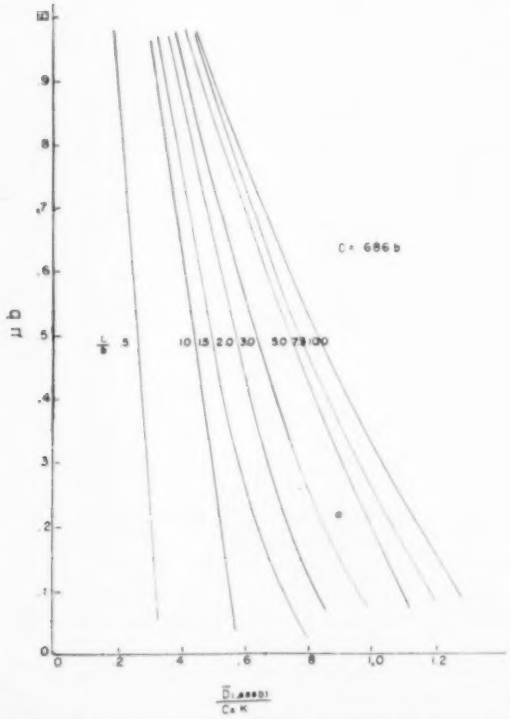

Figure 1. μb vs. average dose rate inside hollow cylinder source at C = .686b.

TABLE 1

Evaluation of $\dfrac{\overline{D}_{(.686b)}}{C_AK}$ for various values of L/b and μb

L/b	μb (.074)	.321	.963
.5	.318	.285	.182
1.0	.550	.485	.291
1.5	.730	.55	.336
2.0	.834	.658	.363
3.0	.97	.745	.394
5.0	1.11	.880	.424
7.5	1.22	.915	.44
10.0	1.29	.95	.445

TABLE 2

Evaluation of $\dfrac{\overline{D}_{(A)}}{C_AK}$ for various values of L/b and μb

L/b	μb .074	.321	.963
.5	.18	.12	.03
1.0	.380	.280	.14
1.5	.550	.390	.175
2.0	.64	.455	.22
3.0	.78	.525	.25
5.0	.945	.66	.29
7.5	1.05	.72	.31
10.0	1.10	.740	.33

Since M is constant:

$$C^2 = \frac{2.62b^2}{C_AK}\,(D_{(C)} - D_{(A)} \qquad (6)$$

where $\overline{D}_{(A)}$ = dose rate at the center line, C = distance along the radius and $\overline{D}_{(C)}$ = average dose rate at distance "C" from center line of source.

This equation was found to fit closely except in outer regions, with dose rate distribution curves found in the literature *(2, 3)*.

TABLE 3

Average valuue for $\dfrac{\overline{D}_{(.686b)} - \overline{D}_A}{C_AK}$ assuming an error of ±.01 units in $\overline{D}_A$ and $\overline{D}_{(.686b)}$

L/b	bμ = .074	bμ = .323	bμ = .963
1.0	.18	.18	.17
1.5	.18	.18	.16
2.0	.18	.18	.16
3.0	.18	.20	.16
5.0	.20	.18	.16
7.5	.18	.18	.15
10.0	.18	.19	.14

It was pointed out *(1)* that the most probable number of survivors in a container could be calculated from the following expression:

$$\int_0^V ndV = \int_0^b n_o\, e^{-(\overline{D}_{(C)}t/D_o)}\, 2\pi hCdC \qquad (7)$$

where $\int_0^V ndV$ = total number of survivors in container

n_o = initial concentration of organisms, no/cc.

$\overline{D}_{(C)}$ = average dose rate at distance from the center line, rep/sec.

t = exposure time, sec.

D_o = specific inactivation dose, rep

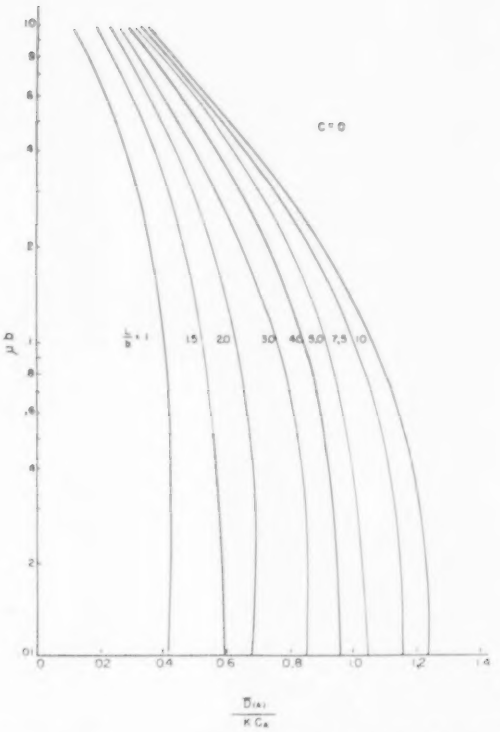

Figure 2. μb vs. average dose rate along center line within hollow cylinder source. (Adapted from Vitro Corporation of America Quarterly Progress Report, April–June 1952.)

h = height of the container, cm.
b = radius, cm.
n = number of survivors
V = volume, c.c.

According to equation (1) $\bar{D}_{(C)} = \dfrac{C^2 + M\bar{D}_{(A)}}{M}$ (8)

By substitution of equation (8) in equation (7) we find that:

$$\int_0^V ndV = hn_o\pi \int_0^b e^{-\left(\frac{C^2 + M\bar{D}_{(A)}}{M} \cdot \frac{t}{D_o}\right)} 2CdC \quad (9)$$

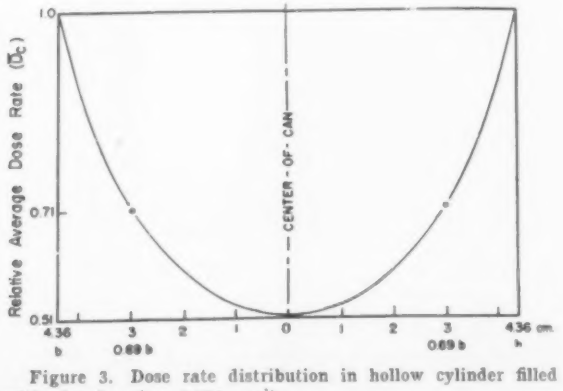

Figure 3. Dose rate distribution in hollow cylinder filled with absorber ($\mu = 0.074$ cm.$^{-1}$).

$$\text{or } \int_0^V ndV = hn_o\pi e^{-(\bar{D}_{(A)}t/D_o)}(MD_o/t)\ [1 - e^{-b^2t/MD_o}] \quad (10)$$

Substitution of equation (4) for M in equation (10) shows that:

$$\int_0^V ndV = \pi hn_o e\left(\frac{-\bar{D}_{(A)}t}{D_o}\right)\left(\frac{2.62b^2D_o}{(C_AK)t}\right)\left(1 - e^{\frac{-C_AKt}{2.62D_o}}\right) \quad (11)$$

As the last term in this equation is negligible in most cases, the final equation becomes:

$$\int_0^V ndV = \pi hn_o e\left(\frac{-\bar{D}_{(A)}t}{D_o}\right)\left(\frac{2.62b^2D_o}{C_AKt}\right) \quad (12)$$

The graphical method has been previously described (1). Equations (2), (3) are solved graphically to determine the dose rate distribution throughout the container, and the dose rate $\bar{D}_{(C)}$ is substituted in equation (7) which is then solved graphically to determine the number of survivors in the container.

The distance along the radius which will have the greatest most probable number of organisms surviving can be determined by letting $\dfrac{d\left(\frac{ndV}{dC}\right)}{dC} = 0$ and solving for $C_{max.}$ (distance measured along any radius from the center line). According to equation (9)

$$\frac{ndV}{dC} = 2\pi hn_o e^{\left(\frac{-C^2 + MD_A}{M} \cdot \frac{t}{D_o}\right)_C} \quad (13)$$

or:

$$\frac{d\left(\frac{ndV}{dC}\right)}{dC} = 2\,hn_o e^{-\left(\frac{D_At}{D_o}\right)} Ce^{-\left(\frac{C^2t}{MD_o}\right)} \frac{2Ct}{MD_o} + e^{-\left(\frac{C^2t}{MD_o}\right)} \quad (14)$$

$$\text{By letting } \frac{d\left(\frac{ndV}{dC}\right)}{dC} = 0 \text{ then } \frac{2C^2t}{MD_o} = 1 \text{ and}$$

$$C_{max} = \sqrt{\frac{MD_o}{2t}}$$

and by substituting $\dfrac{2.62b^2}{C_AK}$ for M, we have the equation:

$$C_{max} = \sqrt{\frac{b^2\,(2.62)\,D_o}{C_AK \qquad 2t}} = 1.146b\sqrt{\frac{D_o}{C_AK}} \quad (15)$$

From equation (15) it appears that the region of the greatest probability of survivors will vary with exposure time, inactivation dose, surface activity of the fission product, radius of the container, and energy level of the fission product.

The basic assumption was made that the classic target theory holds. Although there is some evidence that this is not always the case, it is felt that this assumption is a good approximation for these pur-

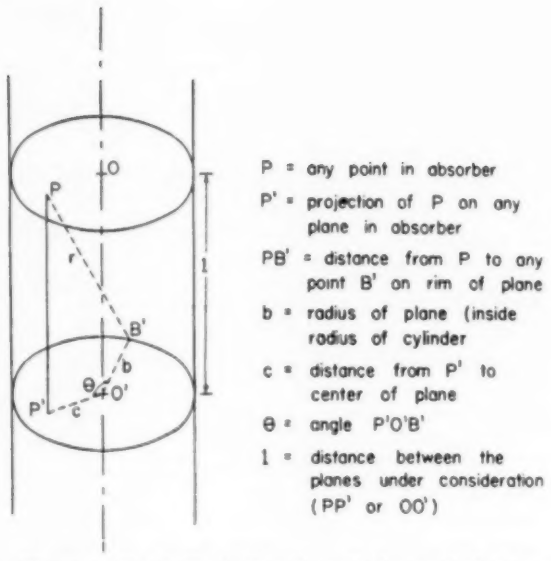
O
P
r
B'
b
θ
O'
P'
c
l
P = any point in absorber
P' = projection of P on any plane in absorber
PB' = distance from P to any point B' on rim of plane
b = radius of plane (inside radius of cylinder
c = distance from P' to center of plane
θ = angle P'O'B'
l = distance between the planes under consideration (PP' or OO')

Figure 4. Sketch illustrating the radiation received by any point "P" in absorber from any point "B" of source.

poses and that slight deviations from the target theory do not appreciably alter the results.

Several examples employing equation (12) to determine the probability of survival in a container are presented in the Appendix (see examples I and II). Close agreement results between the graphical and mathematical procedures. An example is also shown where a dose of 2 x 16^6 rep at the center results in a high probability of spoilage when considering the whole container (see example III Appendix).

In the foregoing procedure, the effect of radiation received as the container approached and left the source has been neglected.

SUMMARY

A simplified equation has been presented for determining the most probable number of survivors in a container irradiated while passing through a hollow cylinder source. This equation is based on the fact that the average dose rate distribution, in the regions critical for calculating most probable numbers of survivors, is a parabola. It has been shown that determining an irradiation process from the standpoint of only dose received at the center may not be theoretically sound. It is suggested that irradiated foods should have the same degree of microbiological safety as thermally processed food.

APPENDIX

Example I

Solution using equation (11):

Given:

$h = 11.58$ cm.
$n_o = 10$ rep/cc.
$t = 30{,}000$ sec.
$D_o = 150{,}000$ rep
$b = 4.36$ cm.
$\mu = 0.074$ cm. $- 1$
$K = 39.7$. Curies to rep/sec. 0.95 m.e.v.
$C_A = 5.05$. Curies/sq. cm.
D_A = dose rate at center
$\mu b = 0.322$
$L/b = 8$
$\overline{D}_A = 39.7\ (5.05)\ (0.72) = 143$ rep/sec.

Problem: Determine probability of survival in container subject to above exposure time. Method of solution:

$$\int_0^V ndV = (11.58)\ (10) e^{-\left(\frac{143(30{,}000)}{150{,}000}\right)} \left(\frac{150{,}000(2.62)19}{(5.05)\ (39.7)\ (30{,}000)}\right) \left(1 - e^{-\left(\frac{(5.05)\ (39.7)\ (30{,}000)}{(2.62)\ (150{,}000)}\right)}\right)$$

$$= 496 e^{-28.6} \frac{250}{200} 1 - e^{\frac{-1000}{2.62}}$$

$$= 620 e^{-28.6} 1 - e^{-381}$$

$$= 620\ (37.3 \times 10^{-14}) = 2.30 \times 10^4 \times 10^{-14}$$

$$\int_0^V ndV = 2.30 \times 10^{-10} \text{ versus } 1.19 \times 10^{-10} \text{ graphically}$$

$$\int_0^V ndV = \text{most probable no. surviving per container}$$

Example II

Conditions the same as in example 1 except exposure time = 6000 sec. Determine most probable number of organisms surviving in container.

Solution:

Using equation (12):

3.9 most probable number surviving per container.

Graphical solution:

4.87 most probable number surviving per container.

Example III

Conditions the same as in example 1 except exposure time is 9.750 sec. Determine most probable number of organisms surviving per container.

Solution:

Employing equation (12):

This exposure time results in a dose at the center of 2 x 10^6 rep.

Most probable number surviving per container is 1.3 x 10^{-2}. This is definitely not a safe process, even though center dose is 2 x 10^6 rep.

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Effect of Wheat Age on Storage Properties of Flour*

(Manuscript received August 16, 1957)

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THE PRACTICE of storing surplus wheat in the United States has prompted questions not only concerning the biochemical and baking properties of flour produced from grain stored for a considerable period, but also about the changes in such flour stored after milling. Although considerable attention has been given to the biochemical changes occurring in wheat during storage *(2, 4, 10)*, the problem of flour changes in storage became of prime technological importance only with the advent of bulk storage. Zeleny has pointed out *(14)* that the main changes occurring in grains during storage are an oxidation of the carotinoid pigments, an increase of acidity and the breakdown of the gluten matrix and starch. It has been established that hydrolytic and oxidative changes in the lipids have an important bearing on the keeping quality of wheat flours *(5, 6, 7, 8, 9, 11, 12, 13)*. Cuendet, Larson, Norris, and Geddes *(6)* found that when various grades of white and whole wheat flour, in a 3% to 14% moisture range, were stored at 100° F., the flours' stability decreased with decreasing refinement, with bleaching, and with an increase in moisture content. The present study was undertaken to develop further information on this problem and to compare particularly the storage behavior of hard winter and spring wheat flours milled after several years' storage with those milled shortly after harvesting.

For this investigation, two classes of bread wheat, namely Hard Red Winter and Hard Red Spring, both of 1952 crop, at various protein levels, were obtained. For comparison, similar wheats from the current crop year (1955) were included. Regular chemical and physical evaluations of the stored patent and straight flours, including a baking test, were made over a storage period of one year at temperatures of 40° and 100° F.

EXPERIMENTAL PLAN

Kinds and sources of wheat

1. *Hard Red Winter Wheats.* Three samples of the 1952 crop Hard Red Winter Wheat varying in protein level, as well as a single sample from the 1955 crop, were obtained. These were commercial wheat types normally supplied to the milling industry. No information was available as to the varietal content, agronomic background or other factors which might influence the quality characteristics. From each of the 8 wheats, 2 grades of flour, namely, patent and straight, were milled on the Kansas State College Pilot Mill. Sufficient flour was obtained to permit 6 testing times at 100° F. and 5 testing times at 40° F. over a one-year interval.

2. *Hard Red Spring Wheats.* Wheats of the spring wheat class consisted of 2 protein levels from the 1952 crop and 2 similar protein levels from the 1955 crop. Each lot of wheat was milled separately into patent and straight grade flours on the College Pilot Mill.

Coding of samples

The following code was used to characterize the various flours:

First symbol:

W if Hard Red Winter Wheat, or
S if Hard Red Spring Wheat.

Second symbol:

I lowest protein level, old wheat, or
II intermediate protein level, old wheat, or
III highest protein level, old wheat, or
C if new crop wheat control. For more than one control, CI, CII, etc. was used to designate protein levels.

* This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense. Contribution No. 289, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station.

Third symbol:

P if patent flour, or
S if straight flour.

Fourth symbol:

H if high temperature storage, or
L if low temperature storage.

Added: Arabic number to indicate month.

Thus: WIIPHS signifies patent flour milled from winter wheat of intermediate protein content stored at at 100° F. for eight months.

Testing schedule

Flour samples stored at 40° F. were tested at the following storage intervals.

3 months,
7 months,
9 months, and
12 months.

Those stored at 100° F. were analyzed at 2 months,
5 months,
6 months,
8 months, and
12 months.

The initial analysis of flours was made immediately after milling (January 3, 1956).

Tests conducted on wheat

All wheat samples were tested as follows:

1. Federal grade, performed by Agricultural Marketing Service, Grain Division, U.S.D.A., Kansas City, Missouri.
2. Germination, performed by Kansas State Board of Agriculture Seed Laboratories.
3. Moisture content.
4. Protein.
5. Ash.
6. Crude fat.
7. Fat acidity.

Tests on flour

Among the tests conducted on flour samples either initially (immediately after milling and prior to storage) or after the various storage intervals, were the following:

1. Moisture.
2. Protein (14% basis).
3. Ash.
4. Crude fat.
5. Fat acidity.
6. Amylograph viscosity (initial only).
7. Farinograph absorption (constant flour weight procedure).
8. Farinograph peak time (dough development time).
9. Farinograph mixing tolerance index.
10. Valorimeter score (a single figure quality score which combines dough development time and mixing tolerance, as obtained by farinograph).
11. Color grade (initial only).
12. Bread score (composite of individual scores) as follows:

Volume	20%
Break and shred	5%
Crumb color	5%
Grain and texture	35%
Absorption	10%
Dough handling	15%
Mixing tolerance	10%
	100%

Tests listed above were conducted according to recognized methods. The majority appear in either *Cereal Laboratory Methods* (A.A.C.C.) *(1)* or in *Methods of Analysis* (A.O.A.C.) *(3)*.

Baking test

During the initial studies with the flour samples, a commercial straight dough formula was used, but this proved to be unsatisfactory, particularly with the spring wheat flours. Accordingly, a sponge dough procedure was adopted with the substitution of fungal enzyme (amylase and proteinase supplement) to replace malted wheat flour. The following sponge dough formula was employed for all subsequent studies:

	Percentage based on weight of flour	*Sponge grams*	*Dough grams*
Flour	100	490	210
Yeast	2	14	
Enzyme (protease and amylase)	(0.3 mg.)	(2.0 mg.)	
$KBrO_3$ (used only in winter wheat flours)	(0.2 mg.)	(1.4 mg.)	
Water	variable	variable	variable
Sugar	5		35
Salt	2		14
Dry milk	4		28
Shortening	3		21

Sponge time, 4 hours. Floor time, 30 minutes. Intermediate proof, 15 minutes.

In the case of spring wheat flours, no bromate was required. All doughs were proofed to a common height (approximately 55 minutes) and were baked for 28 minutes at 425° F.

Milling and storage procedure. After tempering and conditioning in the usual way, the samples representing initial or zero time were tested immediately after milling on the Pilot Plant Mill by normal commercial procedure. Flour produced was stored in 100-pound cotton bags for three days to permit equilibration of moisture in each lot. The flour was then transferred for the storage experiment to five-pound, wide mouth, screw top, glass containers. At each storage interval one or two of these containers were removed and opened for the various analytical tests.

RESULTS AND DISCUSSION

Characteristics of wheats. Data compiled are presented in the form of tables and figures. Table 1 shows analyses on the various wheats conducted prior to the milling test. These data indicate that except for some impairment in the germination in the 1952 winter and spring wheats, the old grains were otherwise normal in character. There appeared to be a slight elevation of fat acidity in the old wheats in comparison with the current crop. Nevertheless, this value in the old wheat is well within accepted limits for normal grain. All wheat samples were of adequately low moisture level, indicating satisfactory storage history. The protein content of the old winter wheat samples was 10.3, 13.4, and 14.9% respectively. The new crop of winter wheat (control) had a protein content of 12.8%. In the spring wheat series, the 1952 crop material contained 12.4 and 13.7% protein respectively, and the new crop samples (1955) showed similar levels, namely 12.6 and 13.5%. All wheat samples showed normal milling characteristics.

Properties of freshly milled flours. Moisture content of the freshly milled flour is indicated under *Moisture* in Table 2 in Column *a*, and that of the same flour after the equilibration period is in Column *b*. The 1952 Hard Red Winter wheats (WI, WII, WIII) produced patent flours with protein content of 9.1,

11.6, and 12.8%, and straight grade flours with 9.5, 11.8, and 12.8% protein. Flours from the 1955 winter wheat (WC) had protein values of 11.2 and 11.4% respectively. The 1952 spring wheat patent flour samples showed protein content of 11.3 and 12.0%, whereas the straight grade protein levels were 11.4 and 12.2%. Control samples of spring wheat flours (1955 crop) showed protein levels for patent of 11.2 and 11.7%, and for straights of 11.8 and 12.1%. Ash levels were normal for commercial flours of this type. However, the 1955 spring wheat series (SCI, SCII) were somewhat lower in ash content than were the similar flours from the 1952 wheats.

Crude fat and fat acidity followed closely the trend in ash in the patent and straight flours. Maximum amylograph viscosity appeared to be inversely related to the protein content; thus the low protein winter wheat (protein 9.1%) gave a viscosity of 810 units indicative of a highly starchy character, whereas the highest protein sample in the group (12.8%) gave a viscosity of 560 units.

Farinograph values appeared to be related to probable flour strength based on protein content. Absorption was related to protein content only in the winter wheat series. Stability to mixing, in terms of mixing tolerance index, decreased with protein content in the winter wheat series, but not in the spring wheat flours.

Spring wheat flours were consistently higher in color grade than the winter wheat flours. The baking value of all flours showed the expected increase with protein content in each series.

Close inspection of all the analytical and baking values obtained at the time of initial testing fails to disclose a significant trend which might be related to age of the wheat.

In the storage experiments which followed, major changes in flour quality appeared to be more closely related to wheat class (i.e. whether winter or spring) than to initial characteristics. For this reason, trends observed for various key quality values are grouped and summarized as averages for winter and spring wheat flours, respectively, and presented in graphs.

Storage of flour at 40° F. Results of various tests conducted during 12 months of storage of the various flour samples at 40° F. may be summarized as follows: Fat acidity showed some fluctuation, but no particular trend in any of the samples throughout the 40° F. storage period. Straight grade flours, as expected, showed an initially higher fat acidity than patent flours, and this difference remained almost without exception throughout the term of the experiment. There was indication of a slight increase in fat acidity in the higher protein flours. In general, however, there was no difference in the trends in fat acidity which could be clearly related to age of the wheat or protein content of the flour, nor did there appear to be significant differences related to spring and winter wheats (see Figure 1).

Absorption values, derived from farinograph curves, showed a slightly increasing trend in the Hard Red Winter wheat flours as storage progressed. In the spring wheat flours this increase was more pronounced. As in the case of fat acidity, however, there did not appear to be any marked relationship between protein content or wheat age with absorption changes. A similar situation prevailed for dough development time in the case of all the Hard Red Winter wheat flours and the older Hard Red Spring material. Surprisingly, however, the patent flours from the 1955 spring wheats exhibited a very marked increase in dough development time; in some cases this value doubled during 12 months of storage. No clear explanation is apparent for this change.

In the case of mixing tolerance index, which indi-

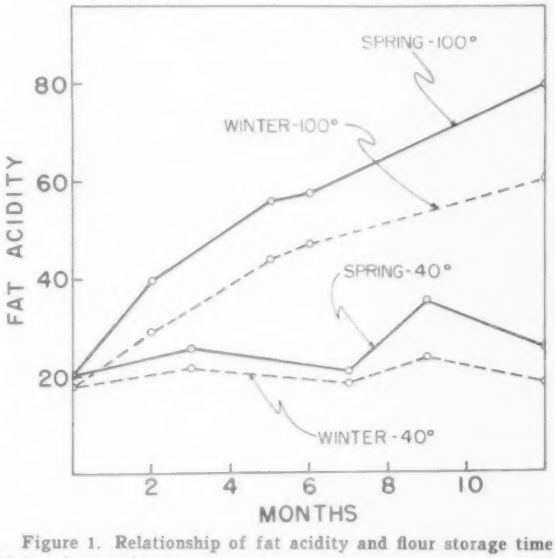

Figure 1. Relationship of fat acidity and flour storage time at two temperatures.

TABLE 1

Properties of wheats used for flour storage experiment

Class	Year	Code	Federal grain inspection				Moisture	Protein	Ash	Crude fat	Fat acidity
			Grade	Test wt.	Total damage	Germination					
				lb.	%	%	%	%	%	%	*mg./100 g.*
Hard	1952	WI	1 Yel. HW	63.0	0.2	71.0	10.6	10.3	1.76	2.25	27.0
Red	1952	WII	1 Dark HW	61.0	1.0	69.0	10.1	13.4	1.84	2.07	20.0
Winter	1952	WIII	1 Dark HW	60.8	1.3	72.0	9.8	14.9	1.87	1.91	22.0
	1955	WC	1 Dark HW	62.2	0.1	81.0	11.2	12.8	1.70	2.03	21.0
	1952	SI	1 hvy. NS	62.3	1.0	75.0	11.4	12.4	1.75	2.1	25.0
Hard	1952	SII	1 hvy. Dk. NS	61.2	1.0	73.0	11.6	13.7	1.72	2.2	20.0
Red	1955	SCI	1 Dark NS	59.3	1.0	86.0	11.0	12.6	1.82	2.3	21.0
Spring	1955	SCII	1 Dark NS	59.6	tr.	87.0	10.6	13.5	1.76	2.2	14.0

TABLE 2

Properties of flour and bread at beginning of flour storage experiment

Flour code	Protein	Ash	Moisture[1]		Crude fat	Fat acidity	Farinograph				Color grade[3]	Loaf volume	Bread score[4]
							Amylograph visc.[2]	Abs.	Peak time	M.T.I.			
	%	%	% *a*	*b*	%	*mg./100 g.*	*B.U.*	%	*min.*	*B.U.*		*ml.*	%
WI P–O	9.1	0.40	12.7	12.2	1.32	18.0	810	62.2	6.5	65	2.0	2607	84
WI S–O	9.5	0.43	12.3	11.7	1.37	19.1	740	62.8	5.0	75	2.9	2650	83
WII P–O	11.6	0.42	12.3	11.6	1.19	15.1	660	67.2	5.5	80	3.0	2750	87
WII S–O	11.8	0.48	11.7	11.7	1.31	16.0	630	65.0	4.0	60	3.7	2850	85
WIII P–O	12.8	0.42	12.2	11.8	1.17	15.1	560	68.0	5.0	90	3.2	2800	86
WIII S–O	12.8	0.48	13.0	11.3	1.14	16.8	580	67.5	4.5	65	3.9	3000	86
WC P–O	11.2	0.40	12.6	12.2	1.08	18.2	580	66.2	6.0	60	2.2	2662	87
WC S–O	11.4	0.49	11.9	11.4	1.28	20.8	580	67.0	6.5	75	3.4	2662	91
SI P–O	11.3	0.42	12.6	11.9	1.22	20.8	600	64.6	6.0	35	1.9	2640	84
SI S–O	11.4	0.44	12.5	12.0	1.24	21.1	495	64.6	7.0	40	2.1	2675	88
SII P–O	12.0	0.40	12.8	12.2	1.06	22.8	545	63.6	7.5	30	1.7	2675	89
SII S–O	12.2	0.49	12.5	12.0	1.33	24.5	540	64.5	5.0	40	3.3	2625	86
SCI P–O	11.2	0.38	13.2	12.6	1.16	18.0	630	60.2	4.0	25	1.3	2575	81
SCI S–O	11.8	0.44	12.5	11.7	1.19	21.1	605	61.6	6.5	40	2.7	2562	82
SCII P–O	11.7	0.37	12.9	12.3	1.22	14.8	610	61.5	7.0	30	1.1	2737	88
SCII S–O	12.1	0.41	12.8	12.1	1.13	17.7	610	62.6	4.5	30	1.7	2725	87

[1] *a* = composite sample after milling; *b* = sample taken for baking after equilibration period and packing.
[2] Maximum viscosity with addition of 0.5% malted wheat flour.
[3] Kent-Jones and Martin Color Grader.
[4] Composite score includes loaf volume, break and shred, crumb color, grain and texture, absorption, dough handling properties, mixing tolerance

cates the resistance of doughs to breakdown with overmixing, the Hard Red Winter wheat materials displayed no clear trend. The 1955 spring wheat flours, however, showed a decrease in this index, indicating the development with storage of an apparently greater resistance to breakdown with mixing.

Combined changes in dough development time and mixing tolerence index are reflected in the valorimeter scores. No trend appeared in these values in the Hard Red Winter wheat flours. However, the trends for the spring wheat flours were mixed. The high protein 1955 material showed a definite increase in valorimeter value in both patent and straight flour; a similar increase in the low protein 1955 patent was also noted. These changes were, of course, reflections of progressive changes in dough development time

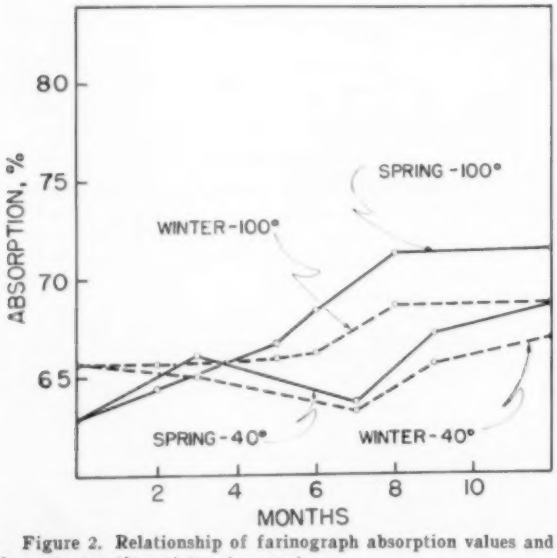

Figure 2. Relationship of farinograph absorption values and flour storage time at two temperatures.

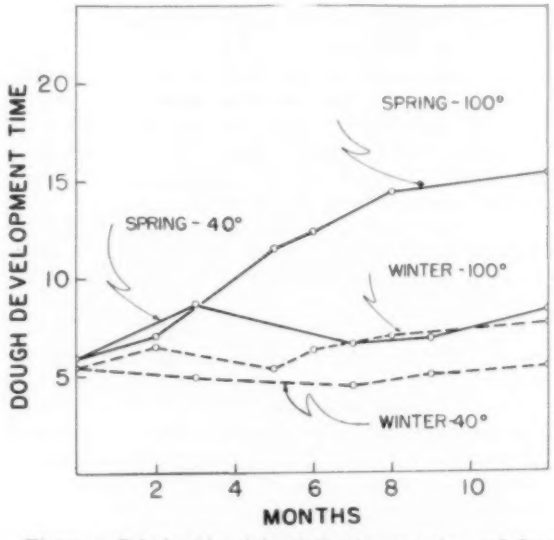

Figure 3. Relationship of dough development time and flour storage time at two temperatures.

and mixing tolerance index. The appearance of some typical farinograph curves is shown in Figure 4.

Bread quality of these series stored at low temperature (40° F.) was evaluated in terms of loaf volume and bread score. There was a fairly definite downward trend during the storage period in both loaf volume and bread score in all the flours from Hard Red Winter wheats. There appeared to be no relationship between this trend and the age of the wheat from which the flours were derived. Most of the spring wheats, on the other hand, showed little, if any, change in loaf volume and bread score, and in a few cases, particularly in material from the new crop, there was an improvement in loaf volume, if not in score.

Storage of flour at 100° F. Changes in flour characteristics with storage at 100° F. were generally

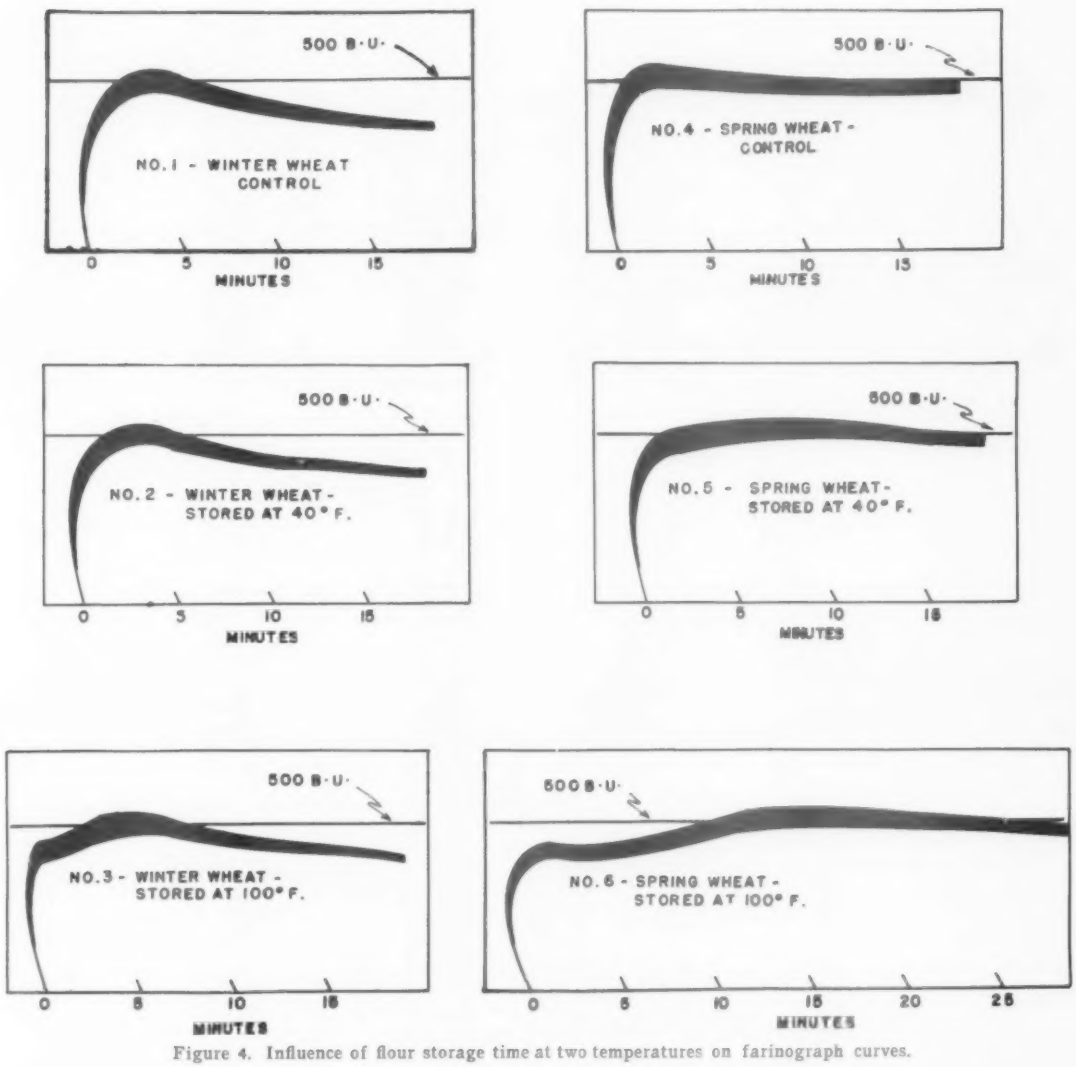
500 B·U·
NO. 1 - WINTER WHEAT CONTROL
0 5 10 15
MINUTES
500 B·U·
NO. 4 - SPRING WHEAT - CONTROL
0 5 10 15
MINUTES
500 B·U·
NO. 2 - WINTER WHEAT - STORED AT 40° F.
0 5 10 15
MINUTES
500 B·U·
NO. 5 - SPRING WHEAT - STORED AT 40° F.
0 5 10 15
MINUTES
500 B·U·
NO. 3 - WINTER WHEAT - STORED AT 100° F.
0 5 10 15
MINUTES
500 B·U·
NO. 6 - SPRING WHEAT - STORED AT 100° F.
0 5 10 15 20 25
MINUTES

Figure 4. Influence of flour storage time at two temperatures on farinograph curves.

greater than at 40° F. In the case of values for fat acidity, a very marked increase occurred in the winter wheat flours to levels 3 or 4 times the initial. Straight grade flours were higher in acidity than patents, but the differences between them remained fairly constant throughout the storage period. New winter wheat flours attained higher fat acidity values than flours from the old wheats. Spring wheat flours increased in acidity more rapidly than the winters, and in most cases, attained considerably higher values after 12 months (see Figure 1). However, in this series, no relationship was revealed between protein content, age of wheat, and rate of development of fat acidity.

Absorption values in all members of the winter wheat series showed some increase, and final values seemed to be proportional to protein content. However, there appeared to be no difference in absorption which could be related to the relative age of the wheat from which the flours were milled.

The stored spring wheat series exhibited a marked elevation in absorption in all samples, with some increases as high as 10%. In this group, as in the winter group, the original age of the wheat did not appear to be a controlling factor. Dough development time showed a consistent moderate increase in the winter wheat flour series and a very drastic increase in the spring wheat flours. In the latter case, dough development times frequently more than doubled (see Figure 3). In spite of these remarkable changes in dough mixing time characteristics with storage, there appeared to be no relationship to the age of the wheat from which the flours were milled. Mixing tolerance index, indicating rate of breakdown of doughs during mixing, showed a decrease in both the winter and spring groups. Some samples displayed this strengthening effect more markedly than others. However, there seemed to be no relationship between this factor and the age or the protein level of the wheat. These mixing changes and alterations in tolerance to

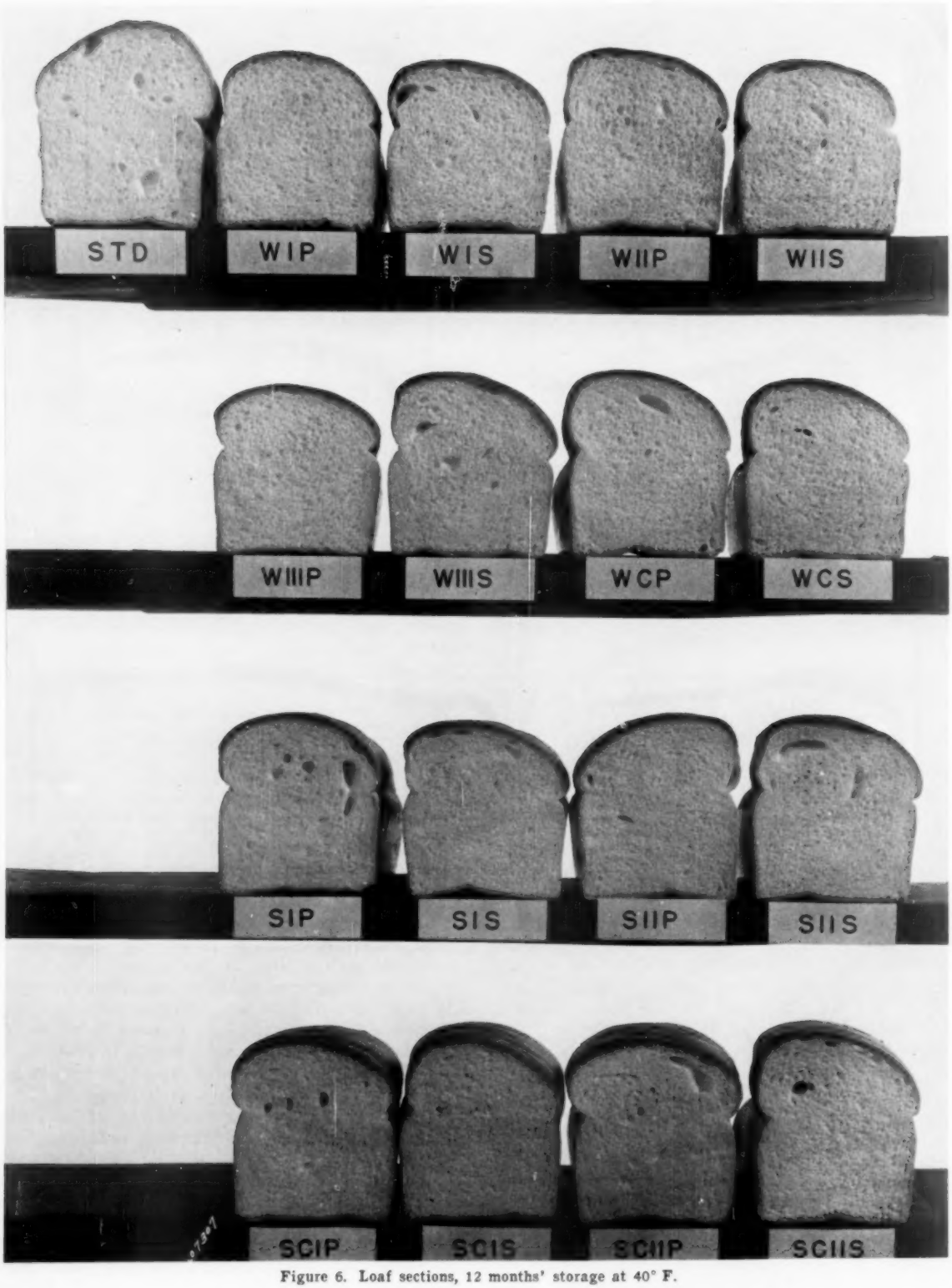

Figure 6. Loaf sections, 12 months' storage at 40° F.

Figure 7. Loaf sections, 12 months' storage at 100° F.

mixing caused a general improvement in valorimeter value of all the flours.

Almost all the winter wheat flours showed marked decreases in loaf volume and bread score when stored at 100° F. By the end of the storage period, the flours indicated impaired dough handling properties, being sticky and lacking in oven spring. These observations are in keeping with those of Cuendet, *et al. (6)*. There appeared to be little difference in this characteristic between the patent and the straight grade flours, nor did wheat age or protein content seem to influence these changes. In the case of the spring wheat flours, loaf volumes and loaf scores decreased much less than in the winter wheats, and in fact, a few of the spring wheat samples showed only negligible changes in loaf volume under these drastic storage conditions. Thus, extensive changes in fat acidity and physical dough characteristics, as observed in this series, affected the breadmaking potentialities only moderately. In any event, no relationship to wheat age and protein content was revealed in any of the changes noted within the range studied. The appearance of loaf sections prepared from all flours after 12 months of storage is illustrated in Figures 6 and 7.

CONCLUSIONS

With reservations, because the various commercial wheats used in this study were inadequately defined as to origin, variety, and agronomic background, and therefore not strictly comparable at the initiation of the flour storage experiment, the following conclusions are warranted:

Spring and winter wheats of the 1952 and 1955 crops used in this study appeared to be sound and representative of commercial materials available when this work was initiated. Milling characteristics were normal, and except for some impairment of germination, the older wheats did not seem to be changed in any way which would affect their technological properties.

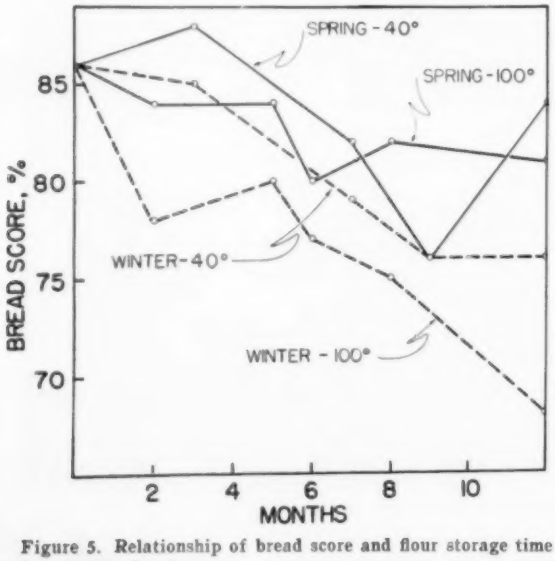

Figure 5. Relationship of bread score and flour storage time at two temperatures.

The spring wheat flours were somewhat lower in ash content and higher in color value than were the winter wheat flours at similar protein levels and extraction rates. Patent and straight grade flours produced on the Kansas State College Pilot Mill showed normal ash values representative of these commercial grades. Amylograph viscosities were normal and inversely proportional to protein content. Farinograph values were also normal for flours of these types. Fat acidity values of straight grade flours were invariably slightly higher than those of patents. The influence of age of wheat was not apparent on these flour properties.

Fat acidity values of patent and straight grade flours milled from winter wheats and stored at 40° F. for 12 months fluctuated in a narrow range, but exhibited no particular trend. The spring wheat flours showed a general increase in this factor. This index of flour quality did not appear to be related to wheat age or protein level.

Changes in farinograph values in flours stored at 40° F. were not found to be related to age of wheat or protein level. Absorption values increased slightly in Hard Red Winter wheat flours and markedly in the spring wheat series. Dough development time increased slightly with storage in the winter wheat flours and strongly in a few of the spring wheat flours. Mixing tolerance improved only in the spring group. Valorimeter values reflected these trends in dough development time and mixing tolerance.

Bread quality in terms of loaf volume and total score deteriorated in all Hard Red Winter wheat flours stored at 40° F. Spring wheat flours showed minor changes in baking quality throughout 12 months of storage at 40° F., and the new crop material showed some improvement in loaf volume, apparently somewhat related to the physical dough changes previously noted. No relationship of bread characteristics to age of wheat was observed, and the loss in bread quality was not paralleled by changes of similar order in fat acidity.

Marked increases in fat acidity occurred in all flours stored at 100° F., the greatest being in the 1955 winter wheat flours and in all the spring wheat flours.

Farinograph absorption values and dough development times increased moderately at 100° F. in winter wheat flours and very markedly in spring wheat flours. Flour grade, wheat age, and protein content were negligible factors in these changes. An apparent improvement in mixing tolerance occurred in all flours. These considerable changes in physical dough properties were not manifested by corresponding improvement in loaf quality. Thus, all the winter series showed a decrease in loaf volume and bread score. Some spring wheat flours evidenced little change in loaf properties with age, and less impairment in dough handling properties than the winter wheat flour series. Increases in fat acidity of the winter wheat flours stored at 100° F., which were accompanied by significant loss in bread quality, were not adequately reflected in the farinograph values. The marked increases in farinograph absorption, dough development

time, and improvement in mixing tolerance noted in the spring series were not similarly related to any corresponding improvements in bread properties, although these held up better than might be expected from the drastic increases observed in the fat acidity.

Differences in age of wheat with apparently satisfactory storage history over the time period investigated in these experiments were essentially without effect on the subsequent rate of deterioration of flours milled from such wheats. In this study, variations in flour grade and protein content produced no special effects other than those normally expected of variables of this kind.

Acknowledgments

The assistance of the Farmers' Cooperative Commission Co., Hutchison, Kansas, and Cargill, Inc., Minneapolis, Minnesota, in securing the wheat samples used in this study is gratefully acknowledged.

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A Consumer Survey versus Panel Testing for Acceptance Evaluation of Maine Sardines

(Manuscript received July 22, 1957)

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IN PURSUING ITS OBJECTIVES of improving quality of sardines *(Clupea harengus)*, the Maine Sardine Council sponsored a consumer acceptance survey and three taste panel tests on the same material. Results showed a rather significant correlation between the panel tests and the consumer evaluations which would appear to be of interest to food technologists as a further step in establishing the status of organoleptic testing as an index of the acceptability of foods.

Although taste panel tests are not considered to be predictive of the response of consumers *(2, 8)*, their goal may be considered two-fold:

(1) The detection of differences among samples as compared with a standard sample. The results are considered as a yardstick to measure differences regardless of preferences.

(2) A guide toward consumer response by means of the preference type of taste tests or even difference tests (if the attributes measured are known to be associated with superior quality or if the standard is known to be of high quality).

There is evidence that, in general, consumers agree with panel findings *(9, 13)* in direction but not in magnitude. In one study *(12)* reference is made to large disagreement between laboratory and field observations. A potato study reported by Harries *(8)* showed that panels detected off-flavors in laboratory samples which dormitory students did not notice when the potatoes were eaten as part of the meal.

Because of expense and technical difficulties, few comparisons have been made between consumer and laboratory results. Most laboratories have to rely on the opinions of relatively small panels.

The primary purpose of the 1955 investigation was to compare the flavors of sardines processed by five relatively new methods with that of fish processed by the conventional method, which is used by 70% of the packers.

Three laboratory taste panels—Maine Agricultural Experiment Station; American Can Co.[c]; and Swift and Co.[d]—independently evaluated the sardines by the techniques which the individual investigators deemed most suitable to the samples. The consumer acceptance study was pursued by a consumer research company.[e]

Objectives:

1. To determine whether or not the flavor of Maine sardines was affected by the method of processing.
2. To compare the findings of the palatability panels with those of a consumer acceptance test, done on identically processed lots of sardines.

EXPERIMENTAL

Materials. It was obvious before the study was begun that variables other than processing method could be controlled only within limits possible under commercial conditions. The 6 packs had to be processed at the different factories equipped for the specific method involved, so that the fish were not from the same seine and probably not from the same section of the Maine coast. In addition, they were not comparable as to the amount of salt added to the fish on the boats, time lapse from catching to factory, and from delivery to processing.

The fish were all packed in soybean oil, but it was not possible to use the same batch of oil for all methods as the factories were at a considerable distance apart.

Two of the samples, numbers 23 and 37, acquired a slight flavor of spice from other operations in the processing plant in spite of extreme care to preclude it. The flavor, of course, is not normal for any commercial sardine and was not intended to be measured in the study.

Thus it was realized that differences *between* samples could be caused not only by processing method but might also be attributed to source of fish, salt content, source of oil, or spice flavor. The samples *within* a processing method were completely uniform, however. They were processed at the same time under exactly the same conditions and were of extra standard quality averaging 10 sardines to the can.

The fact that 2 samples were known to have a vague spiced flavor did not affect the consumer survey results on the other 4 samples because the samples were presented in pairs (only one pair to a family). One member of the pair was always from the conventional processing method which was considered the standard sample. Because two of the samples were known to be different, it seemed an ideal opportunity to compare taste panel results with the consumer survey.

The methods of processing are not presented here because they are not pertinent to the purpose of this report, which merely compares taste panel findings with each other and with a consumer survey. In addition, the methods are considered irrelevant because the between-sample variability was such that confounding of effect with causes other than processing method was possible.

The codes for the 6 treatments were:

53 (standard conventional)	41
82	37
59	23

The following report is a detailed description of the palatability test as pursued at the Maine Agri. Expt. Sta.

Method and design of experiment. Forty-three tasters judged the 6 samples by a ranking method *(1)*, a technique adapted to untrained judges and classified as a preference type of test.

Samples were presented in a balanced, incomplete block design *(4)* which had the following characteristics:

No. of samples	6	No. of blocks	10
No. of samples in block	3	No. of times two samples	
No. of replicates	5	appeared together	2

[a] Horticulture Department, Maine Agri. Expt. Sta., Orono, Maine.
[b] Maine Sardine Council Research and Quality Control Laboratory, Bangor, Maine.
[c] Taste tests supervised by Mardell D. Tompkins, American Can Co.
[d] H. R. Vernon, Supervisor, Chicago Laboratory, Swift & Co.
[e] Batton, Barton, Durstine and Osborn, Inc., New York, N. Y.

Presentation of blocks and the order within blocks were randomized.

Tasters were asked to rank on the basis of flavor only each of the 3 samples in order of preference, with the one he liked best in first place and the one he liked least in third place. He was also asked to check whether or not each sample was acceptable (Figure 1).

Maine

RATE FLAVOR ONLY. IGNORE ALL OTHER DIFFERENCES

Name:_____ Product:_____

Date:_____ Block No:_____

Instructions: Rank the samples with the one you like best in first place and the one you like least in last place. No ties should be given.

	Write sample number Flavor	Check Are samples acceptable? Yes	No
1st (best)	_____	_____	_____
2nd	_____	_____	_____
3rd (poorest)	_____	_____	_____

Figure 1. Flavor evaluation.

In this kind of taste test, an attempt is made to rule out any extraneous factors (except the one under consideration) which could possibly influence the judges. Appearance and color is disguised as much as possible to preclude undue influence. On the other hand, it is desired, as nearly as possible, to maintain the identity of the food in the condition that it is usually eaten.

It is known that sardines, as packed, retain the viscera, including a gall bladder which imparts a bitter flavor. To ordinary consumers who eat whole fish, this small amount of bitterness may be unimportant. To judges who are consciously attempting to differentiate flavors, a small amount of bitterness may be detectable and sway their judgment for or against a certain sample. To preclude this influence, the sample sardines were all eviscerated. The backbones were also removed to eliminate that difference in texture from affecting the judges' decisions.

Four cans of sardines were used for each sample. They were drained of oil in stainless steel colanders for 5 minutes, eviscerated, filleted, cut into bite-size pieces and mixed.

Samples were served, coded, on white glazed plates, three at a time. The 10 blocks were served with at least 20 minutes between samples during one day—some in the morning and some in the afternoon.

Judges. Most of the judges were experienced in judging foods but none had served on panels to judge fish of any kind. All were employed in academic work, teachers, research staff, clerks and graduate students. The panel comprised 9 women and 34 men. Of the group, 24 were Maine natives, 5 were from other parts of New England, 13 were from various other parts of the United States, and one was from Mexico.

Statistical treatment. The record of each judge was analyzed by a quick variance method *(15)* which showed that 36 of the 43 judges detected significant (5% level or better) flavor differences among the samples.

The ranks were transformed to scores (+.85, 0, −.85) according to Fisher and Yates *(6)* for normalizing ranked data, and the combined scores for all judges were analyzed by the variance method.

RESULTS

Analysis of variance of the scores of all 43 judges showed that the judges had significant (1% level) preferences among the samples (Table 1) which were associated with processing methods. The four samples, 53, 82, 59 and 41, were not significantly different from each other although the scores ranged from +61 to +30 *(5)*. Samples 23 and 37 were significantly low in the array of scores but were not different from each other.

In other words, the first four were highly preferred to the other two.

The American Can Co. test was done in complete blocks of 6 samples of whole drained fish by a selected panel of 10 discriminating people from among 16 tested. Judge acuity was determined from decisions on duplicate samples of the sardines. In the final test judges were asked to rank the 6 samples in order of preference. The presentation of samples was randomized and the test was repeated three times.

Choices were assigned scores of 1 *(best)* to 6 *(poorest)* and the data were tested for significance by Friedman's chi square *(7)*. Total scores and significance levels are summarized in Table 1. Final results indicated a significantly higher preference for samples 59, 82, 41 and 53 than for samples 37 and 23. Within the four preferred samples and the two less preferred, there were no significant differences.

A third panel test, done by Swift and Co. with 24 judges, resulted in similar preferences. This panel tested whole fish as

TABLE 1

Sardine palatability as associated with six processing methods evaluated by three taste panels

Me. Agri. Expt. Sta.

Sample No.	Total transformed scores ranked 43 judges [2]
82	+61.20
41	+51.85
59	+47.60
53 [1]	+29.75
	----- [3]

37	−85.00
23	−105.40
L.S.D. 5%	36.25
1%	47.81

American Can Co.

Sample No.	Total scores [4] ranked [1 = 1st choice, 6 = poorest] 10 selected judges
59	67.0
82	79.5
41	79.5
53 [1]	84.5
	---- [3]

37	159.0
23	160.5

Swift and Co.

Sample No.	Average scores [4] ranked [1 = 1st choice, 3 = poorest] 23 judges
41	1.7
53 [1]	1.7
59	1.7
	... [3]
82	1.9
	... [3]
	...
37	2.5
23	2.6

F table

Source	D.F.	M.Sq.	F
Treatment	5	25.97	32.87**
Treat x Judge (error for treat)	210	0.79	2.47**
Error	1032	0.32	

Chi square [5]

Formula $X^2_r = \frac{12}{np(p+1)} \sum_{i=1}^{p} \left(\sum_{j=1}^{n} r_{ij} \right)^2 - 3n(p+1)$

where n = number of tasters
p = number of samples
r_{ij} = rank of sample, i, by taster, j

F table

Not available

[1] Standard sample = conventional pack.
[2] 36 significant (5% or better).
[3] Broken lines indicate significant difference in scores (single p = .05; double p = .01).
[4] Lowest score indicates highest preference.
[5] Data tested for significance by Friedman's chi square *(7)*.

they come from the can after draining the oil. Samples were presented in a randomized balanced incomplete block design, 3 at a time, in the same design that was used in the Maine study (see above). The ranks were normalized by transforming to scores *(6)* and were analyzed by the analysis of variance. Results were reported as average ranks (1 to 3) (Table 1). The panel indicated that samples 41, 53 and 59 were preferred significantly to samples 37 and 23 as well as to sample 82. The F Table was not available to us.

It would seem that all 3 panels agreed on the undesirability of samples 37 and 23 and that only one panel indicated that sample 82 was less desirable than the three other samples.

Since the presence of widely different samples makes others seem more alike, the 4 samples which were not significantly different, viz. 53, 59, 41 and 82, were retested at Maine omitting the two samples which were obviously unpalatable (23 and 37).

For this test the paired comparison method of Scheffé *(11)* was used. Previous work has indicated *(10)* that smaller differences may be detected by this technique than by ranking or by checking descriptive terms. This method involves presenting paired samples in a completely balanced incomplete block design. The judge is requested to check the sample which he prefers and to indicate the degree of difference between the pair members (Figure 2).

Twenty-two judges were randomly selected from among the 36 significant judges in test 1. Eleven of these judges were selected to judge the samples presented in one direction while the other eleven judged the samples presented in the reverse order. The test was replicated 4 times with 3 pairs presented at one test session.

The resulting data were analyzed in two ways. The simple preferences were handled as ranked data, transformed to rankits *(6)*, and submitted to analysis of variance (Table 2). By this method and using the interaction mean square (treatment × judge) as error, there was shown to be no significance attached to the treatment differences although sample 59 was preferred (10% level) to sample 53. If conclusions are limited to the panel only, using error M. Sq. to determine treatment F, sample 59 would be considered preferred to 41 and 53. For predictive purposes, however, the differences would be considered not significant.

A second analysis, according to the Scheffé modified analysis of variance using the records on degree of difference (intensity scale) (Table 2), showed that sample 59 was significantly ($P = .05$) better than samples 41 and 53. Otherwise the between-sample differences were not significant.

A review of objective tests done on these samples seemed to show an association between salt content and sample preference (Table 2). It was also observed that from among the 22 judges, 6 noted sample 59 as salty; 3, sample 82; 2, sample 41; and 1, sample 53.

CONSUMER ACCEPTANCE TEST

The objective of the consumer test was to obtain information on consumer preference between conventional oil-packed sardines and other types of packs. It included:

Over-all preference between conventional and the other packs, after tasting two sardine samples randomized for order of presentation.

The "in-home" taste tests were conducted in 12 cities in each of which 350 families were visited. Preference information was obtained from 5143 sardine consumers for the pairs considered in the study. In each family, *only one* of the trial samples was compared to the conventional. An equal number of families participated in *each* of the tests in *all* cities.

The pertinent section of the record form is included (Figure 3).

NOTE: Give the respondent a taste of Product No._____

7. What is your opinion of this can of sardines?

Like very much (___) Dislike somewhat (___)
Like somewhat (___) Dislike very much (___)

NOTE: Give the respondent a taste of Product No._____

Like very much (___) Dislike somewhat (___)
Like somewhat (___) Dislike very much (___)

8. Taking everything into consideration, which of these 2 products do you prefer?

NOTE: Respondents may retaste sardines if they wish.

No._____
Product No._____ very much better (___)
Product No._____ somewhat better (___)
No._____
Product No._____ very much better (___)
Product No._____ somewhat better (___)
No difference (___)

Figure 3. Questionnaire form for consumer acceptance.

Final data were reported in percentage of preferences of the conventional versus the other member of the sample pair in 3 degrees of intensity: *no difference, like somewhat better,* or *like very much better.* The percentages were reduced to "number of judges" in order to submit the data to an analysis of variance *(3)*. The degrees of difference were assigned transformed scores of 0 for conventional with ±.50 for *liked somewhat better* and ±1.16 for *liked very much better (6).*

Although equal numbers of families in each city tasted each pair, varying numbers of family members gave unequal judge numbers per city. Therefore, the scores were averaged before analysis *(3)*. The range of judge numbers varied from: 1000 to 1070 per treatment pair and from 32 to 109 judges per city per treatment.

The analytical results are shown in Tables 3 and 4.

The mean scores for all cities indicated that the consensus of consumer opinion and that of the three panels followed the

Scoring Method: D **Maine**

RATE FLAVOR ONLY. IGNORE ALL OTHER DIFFERENCES

Name:_____ Product:_____

Date:_____ Block No:_____

Instructions: Compare each pair separately. Taste in order: Pair 1, 2, 3. Always taste sample A before sample B.

	Check Preference		Check one The difference is:			
	A	B	Large	Moderate	Slight	None
Pair No. 1						
2						
3						

Figure 2. Flavor evaluation.

TABLE 2

Sardine palatability as associated with four processing methods determined by a paired comparison technique (Scheffé), Maine Agri. Expt. Sta.

Sample no.	Ranking Preferences only[1] 4 replicates 22 judges	Sample no.	Intensity scale[2] 4 replicates 22 judges	Per cent Na Cl Ave. 20 samples
59	+29.12	$59\text{-}\hat{a}_1$	+0.2586 [3]	2.28
82	+ 3.36	$82\text{-}\hat{a}_2$	+0.0142 [3]	2.04
41	−14.56	$41\text{-}\hat{a}_3$	−0.0994	1.27
53	−17.92	$53\text{-}\hat{a}_4$	−0.1733	1.24
L.S.D. 10%	40.59			
5%	not significant	Y .05	0.3210	

Source	D.F.	M.Sq.	F	Source	D.F.	M.Sq.
Treatment	1	1.76	1.57	Main effects	3	12.55**
Judge x treat (error for treat)	63	1.12	4.31**	Deviations from subtractivity	3	2.20
Tr x presentation	3	0.30	1.15	Order effects	6	3.73
Error	961	0.26		Error	516	2.66

[1] Preferences transformed to scores (*6*) and analyzed by variance.
[2] Degree of preference between pairs analyzed according to Scheffé (*11*).
[3] Bracketed scores do not differ significantly.

same pattern of flavor preferences. The differences between sample 53 and samples 59, 41 and 82 were not detectable to a significant degree but a trend favored sample 59 over sample 53. Samples 37 and 23 were considered to be less palatable (P = .01) than sample 53. The responses of the various cities were different (P = .01) but when all treatments were considered together, only Wheeling demonstrated a significantly divergent opinion. Analyses of the responses of cities to each treatment pair (Table 4) show the reaction of the different cities to the processing methods.

TABLE 3

Consumer preferences for sardines
Paired comparisons in 12 cities of five processing treatments against the conventional method (sample 53)[1]

Treatments	Mean scores all cities[2]	City	Mean scores all treatments[2]
59	+.14	Tucson	+.07
		Los Angeles	+.06
41	+.01	Duluth	−.03
		Des Moines	−.07
(53)	0	Jackson	−.07
		Philadelphia	−.08
	−.02	Tacoma	−.13
82	----	Detroit	−.13
	----	Bridgeport	−.15
37	−.39	Richmond	−.17
23	−.40	Dallas	−.23

		Wheeling	−.63
L.S.D. 5%	.19	L.S.D. 5%	.29
1%	.25	1%	.39

Source	D.F.	M.Sq.	F
Treatment	4	.72	14.40**
Cities	11	.16	3.20**
Error	44	.05	

[1] Sample 53 always considered as 0.
[2] Broken line indicates significant difference in scores (P = .01).

DISCUSSION

A comparison of effects of processing methods on the flavor of Maine sardines as determined by 3 independent taste panels and by a consumer acceptance study showed essential agreement among the panels and with the survey. Conclusions from the taste panel studies were parallel to those from the acceptance study and could have predicted rather closely the preferences of the consumers.

Two deviations from this general pattern occcurred. The Swift and Co. panel of 24 judges indicated that sample 82 was significantly poorer than conventional. The other two panels and the consumer survey found no significant differences between sample 82 and sample 53. In a second test, with 22 selected judges and by a sensitive paired comparison technique, the Maine Experiment Station panel indicated that sample 59 was significantly preferred to samples 53 and 41. This

TABLE 4

Consumer preferences for sardines
Comparison of paired processing treatments by city. Conventional (sample 53) method always considered as 0

53 vs. 37 City	Mean score[1]	53 vs. 23 City	Mean score	53 vs. 59 City	Mean score	53 vs. 41 City	Mean score	53 vs. 82 City	Mean score
Bridgeport	+.18	Tucson	−.01	Tucson	+.57	Los Angeles	+.57	Duluth	+.30
Philadelphia	+.02	Philadelphia	−.14	Los Angeles	+.54	Detroit	+.15	Los Angeles	+.22
Des Moines	−.30	Jackson	−.15	Richmond	+.38	Tacoma	+.09	Tucson	+.21
Detroit	−.33	Des Moines	−.25	Des Moines	+.29	Jackson	+.09	Tacoma	+.13
Duluth	−.34	Bridgeport	−.26	Duluth	+.27	Richmond	+.06	Richmond	+.12
Jackson	−.38	Los Angeles	−.33	Tacoma	+.23	Des Moines	+.02	Jackson	+.05
Dallas	−.42	Duluth	−.38	Dallas	+.10	Tucson	+.01	Detroit	+.01
Tacoma	−.42	Detroit	−.54	Detroit	+.04	Duluth	−.02	Des Moines	−.11
Tucson	−.43	Dallas	−.58	Jackson	+.03	Philadelphia	−.05	Dallas	−.18
Los Angeles	−.71	Richmond	−.65	Philadelphia	+.03	Dallas	−.07	Philadelphia	−.27
Wheeling	−.73	Tacoma	−.66	Bridgeport	−.23	Bridgeport	−.15	Bridgeport	−.30
Richmond	−.78	Wheeling	−.80	Wheeling	−.55	Wheeling	−.61	Wheeling	−.47
L.S.D. 5%	.25	L.S.D. 5%	.23	L.S.D. 5%	.24	L.S.D. 5%	.25	L.S.D. 5%	.27
1%	.33	1%	.31	1%	.32	1%	.32	1%	.35

[1] Bracketed scores do not differ significantly as determined by Duncan's method (*5*). This was an approximate comparison using a calculated average number of judges per city (*14*).

conclusion also was not confirmed by the large consumer group. Sample 59 [1] was rated first by the consumers but was not significantly preferred to sample 53. In both cases the panels were apparently more acute than the consumers and drew a finer distinction among the samples than could be drawn by the consumer group.

Although this study does not prove that taste panels will predict consumer preferences, it does lend support to the idea that panels have some predictive value and are not a "thing apart." In the two instances where the panels diverged from the consumer opinion, the panels' judgment was on the more critical side. From the viewpoint of the food industry the divergance would be more serious had it predicted an acceptance of samples which would be rejected by the consumer. The study also indicated that the less sensitive methods (e.g. ranking) more nearly predicted consumer preferences than the more precise paired comparison technique.

A great deal more evidence is needed to fully support the idea of a close association between panels and consumers, using samples with smaller flavor differences than were found between the sardine samples.

SUMMARY

The effect of five different processing methods on the flavor of Maine canned sardines was tested organoleptically by three independent taste panels and by a consumer preference survey against a standard sample processed by the so-called conventional method.

The three taste panels used the ranking technique to judge the samples: (1) whole drained fish in complete blocks of 6 samples with 3 replicates by 10 judges selected for acuity; (2) whole drained fish in balanced incomplete blocks of 3 samples with 5 replicates by 24 unselected judges; (3) drained, eviscerated, filleted fish cut in bite sizes in balanced incomplete blocks of 3 samples with 5 replicates by 43 experienced but unselected judges.

The consumer acceptance survey was done in 12 cities with equal numbers of families (but not always equal numbers of consumers) tasting sardines canned by the conventional method and those processed by one other method. The total number of tasters per pair of samples varied from 1000 to 1070. The consumer indicated his preference for one member of a pair and the degree of difference.

Data from the panel studies and those from the consumer survey were submitted to analyses of variance except for the data from the American Can Co. panel which were analyzed by a chi square method.

All panels and the consumers detected significant differences among the samples and all agreed that samples 59, 41, 82 and 53 (standard) were significantly preferred to samples 37 and 23. One panel only indicated that sample 82 was significantly poorer than 59, 41 and 53.

The four highest ranking samples were retested by one taste panel (22 judges selected for acuity) by the Scheffé paired comparison method. Variance analysis of the simple preferences indicated no difference among the samples. Analysis of the degrees of difference between pairs showed that sample 59 was significantly better than samples 41 and 53. This difference was not detected by the consumer group when the analysis was based on the mean scores.

[1] It may be of interest to note that the original interpretation of the consumer study, based on percentages alone, considered sample 59 to be preferred to sample 53. Variance analysis showed that there was no significance attached to the difference between the mean values.

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The Effect of Various Additives on the Stability of Cured Hams[a,b]

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ADDITION of ascorbic acid or sodium ascorbate has been shown to speed normal color development in meat and meat products *(2, 7, 15)*. This is accomplished by the reduction of metmyoglobin and nitrite to myoglobin and nitric oxide. These compounds then react to form the characteristic "cured meat pigment" nitric oxide myoglobin *(2, 8)*. Ascorbic acid has also been shown to protect cured meat color from fading *(15)*. With the reduction of the nitrite and metmyoglobin assured and with fast penetration of the curing ingredient into the ham tissue, curing time could be reduced.

Hydrated calcium lactate and lactic acid, when used in conjunction with the other curing ingredients, has been reported to accelerate salt penetration *(14)*. Weak acid and the salt of the weak acid form a metallic-acid ion in solution which serves as an accelerator to speed penetration of the sodium chloride and other salts into the meat tissue. This metallic-acid ion also acts as a buffer and stabilizes the acidity of the solution at a pH value between 5.8 and 6.3.

In recent years phosphates have been added to meats in an effort to increase desirable texture, uniform color, and juiciness. Hams cured with phosphates included in the curing mixtures have appeared to be firmer and to have less free moisture than control hams *(16)*. Disodium phosphate when incorporated into the cure has been shown to improve substantially the resistance to discoloration and to improve the appearance and flavor *(1)*.

Ascorbates and nitrites are quite reactive at low pH value or at high temperature. It has been shown, however, that these compounds are rather stable in high concentration of salts, in a pH range of 6.5 to 7.0 when stored under refrigeration *(8)*. Since a normal brine solution is in this pH range or above, ascorbates and nitrites can be incorporated in the chilled brine without reacting to form nitric oxide gas.

The per cent of trimmed ham weight to the carcass weight is remarkably uniform within grades of hog carcasses ($r = .99$) (Missouri, unpublished data). Therefore, it is possible to calculate the ham weight before it is removed from the carcass. It is feasible to inject the hot ham before cutting it from the carcass, since the femoral artery is readily available. This would allow the curing process to proceed during the normal chilling time after slaughter, and thereby, to shorten the process time.

An increase in temperature during the curing operation has also been shown to accelerate greatly the curing process *(6, 9, 11)*. The temperature of the hot carcass would aid in speeding up the curing reaction.

Objective of this investigation was to study the effect of sodium ascorbate, hexametaphosphate, and calcium lactate lactic acid mixture incorporated in the curing solution on cure penetration, color development and stability, and palatability factors in ham injected before chilling and after chilling.

MATERIALS AND METHODS

Experiments were carried out using both hot and chilled carcasses from 32 hogs which weighed approximately 200 pounds. Carcasses graded U. S. 1 and U. S. 2 according to the Federal grade specifications for barrow and gilt carcasses. The ham on the right half of each carcass was injected hot before removing it from the carcass. Weight of the ham was determined by multiplying the hot carcass weight (packer style) by 18 and 19% for U. S. 2 and U. S. 1 grade carcasses, respectively (U. of Mo., unpublished data). The left ham was removed and injected after chilling 24 hours at 34–36° F. All hams were injected with curing solution equivalent to 10% of calculated green weight and actual green weight for the hot and chilled hams, respectively. Control hams were injected with a sterile 75° salinometer pickle made of salt, cane sugar and sodium nitrite. Salt and sugar were in the ratio of 7:3, respectively; 9.08 g. of nitrite was added to the diluted brine (75°) per gallon. This solution was diluted to 65° salinometer reading for the cover pickle on the control hams. Control hams remained in cure for 4 days.

Sodium ascorbate was added to the above curing mixture in the amount of 2.13 g. per gallon. This mixture was injected into both the hot and chilled hams in the ascorbate treatment.

Just prior to injecting the cure into the hams, 2.13 g. of sodium ascorbate and 8 ounces of sodium hexametaphosphate were added to the regular curing mixture. This mixture was injected into both hot and chilled hams receiving ascorbate-phosphate treatment.

Hams to be treated with calcium lactate-lactic acid were injected with a 75° salinometer pickle prepared from a commercial mixture[f] containing sodium chloride, hydrated calcium lactate, 85% lactic acid, cane sugar, sodium nitrate and sodium nitrite.

A dry cover cure of the same ingredient in the same proportion as used in the pump pickle was applied to the treated hams. Hams injected after chilling were rubbed with the cover cure at the time of injection. The cover cure was on these hams for 48 hours. Hams which were injected hot had the cover cure applied when they were removed from the carcass. Therefore,

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[f] Supplied by Koch Mfg. Co. through the courtesy of R. H. Starr, Kansas City, Missouri.

the cover cure was on the hot injected hams for 24 hours. All the treated hams were cured for 48 hours after they were injected.

At the end of the curing time all hams were smoked and heated to an internal temperature of 142° F. Treated hams were held in the cooler (40° F.) until control hams were ready for evaluation so that evaluation could be made simultaneously. A commercially cured ham was purchased for each trial for comparison with the treated and control hams.

Chilled hams were cut and a center slice removed for ascorbic acid determination. The procedure of Hoffman-La Roche, Inc. *(12)* was used for ascorbate determination.[5] A qualitative test for nitrite was determined by using Griess sreagent on filter paper on the ham slice *(10)*. Two-tenths ml. of a sulfanilic acid-acetic acid solution, (10 g. of sulfanilic acid in 1000 ml. of 30% acetic acid) and 0.2 ml. of α-naphthylamine-acetic acid (0.03 g. of α-naphthylamine in 70 ml. of water added to 30 ml. of glacial acetic acid) was dropped on nitrite negative filter paper and the filter paper was pressed onto the surface of the meat. A pink color on the filter paper denoted residual nitrite. Depth of color was proportional to the amount of nitrite *(4)*. Another center slice was removed from each ham and packaged in 300 LSAT cellophane. These slices were displayed at 38–40° F. under 30 foot-candles of light with an air flow of 15 lineal feet per minute. Subjective color comparisons were made by members of the U. of Mo. Meat Section Staff over a 48 hour period at 24 hour intervals. For comparison, a fresh cut ham slice was removed from each ham and packaged just prior to color evaluation. Color rating was based on a hedonic scale ranging from 1, *very undesirable*, to 8, *very desirable*. Uniformity of color was also judge visually and ranked on a similar hedonic scale.

A third slice, one inch in thickness, was removed from each ham for organoleptic comparisons by a panel of 6 members. These ham slices were cooked to an internal temperature of 160° F. in a 300° F. oven. Each member of the panel was served a warm sample taken from the same relative position in the ham slice throughout the experiment. Evaluations were made for saltiness, flavor, juiciness and tenderness using the hedonic scale method. Number 1 on the scale was *very undesirable;* number 8 was *very desirable*.

Analysis of variance *(13)* and multiple range test *(3)* were used to test for significance differences between treatments.

RESULTS AND DISCUSSION

The calculated trimmed ham weight from the hot carcasses averaged 14.26 ± .64 pounds as compared to the 14.28 ± .72 pounds for the actual weight of the hams from the chilled carcasses. Therefore, the amount of cure injected into the right ham while hot was the same as that injected into the left chilled hams. No difficulty was encountered in injecting the curing solution into the hot hams through the femoral artery. The remainder of the carcasses was handled routinely as very little of the injected cure flowed into the loin or belly.

A summary of mean panel score values and standard errors on the factors evaluated in the different treatments is given in Table 1.

Uniformity of color. The variation in uniformity of color in the center ham slices is shown in Figure 1. There was considerable difference between the uniformity of the commercial ham slices and that of the treated or control ham slices. The outer periphery of the *semimembranosus* and the *semitendinosus* muscles were generally lighter in color than the remainder of the ham slice. This was observed rather uniformly among short cured hams. This is in agreement with observations made by others *(7)* and is thought to be due to (a) lack of myoglobin in this area and/or (b) lack of migration of cure into these muscles near the outer periphery. Sodium ascorbate and/or sodium hexametaphosphate or calcium lactate did not result in any marked improvement in cure penetration or uniformity of color in these areas. However, as shown in Figure 1, those hams injected hot had slightly higher panel scores on uniformity of color than paired hams injected with the same solutions after being chilled. This would indicate that cure penetration was somewhat better in the hot injected hams but not enough to be significantly different. Control hams were as uniform is color as any of the hot or chilled treated hams, which would indicate the additional time in cure alleviated any beneficial effect of increased temperature on cure penetration in the hot cured hams. All of the hams ranged slightly desirable or higher in uniformity as judged by the panel.

Color stability. All of the ham slices discolored very rapidly when displayed under 30-foot candles of light at 38–40° F. in the display case. Rate of discoloration was about the same in the treated hams as in the control hams and the commercial hams. At the end of 24 hours of display time, all the slices were ranked as moderately undesirable by members of the University of Missouri meats staff. This does not confirm previous work reporting that ascorbic acid, sodium ascorbate and/or sodium hexametaphosphate aids in fixing and maintaining a desirable color in cured meats when incorporated into the curing mixture *(7)*.

There was no appreciable difference in the stability of color in either of the treated samples. Tests were used to determine if residual nitrite and/or sodium ascorbate was present in the fresh cut and the displayed ham slices. Nitrite was found in all the ham slices even after 24 hours of display time. However, no ascorbate was found in any of the ham slices when analyzed immediately after cutting. This indicated the sodium ascorbate had been completely oxidized in the curing and smoking process. Therefore, none was available for the reduction of nitrite and/or metmyoglobin in the display slice. This was verified by spraying the ham slice with 5% sodium ascorbate solution. After 10–12 hours in the display case unexposed to direct light the color regenerated to the normal color of a fresh cut slice. Display ham slices were also dipped after 24 hours in a solution of nitrite and sodium ascorbate in the same proportion as in the curing mixture. Color regenerated in those slices dipped in the above solution to the normal color of a fresh cut slice in about the same time as in those sprayed with a 5% ascorbate solution. Freshly cut slices were sprayed with a 5% solution of sodium ascorbate before being placed under the light. These slices maintained a much brighter color than those not sprayed under the same conditions. This is in agreement with results of other researchers *(7, 10)* using ascorbic acid solution as a spray to maintain a desirable color in cured meat. The above results indicate that ascorbic acid or sodium ascorbate are not bene-

[5] Dissolve approximately 135 mg. sodium 2-6-dichlorobenzenone indophenol in 250 ml. hot water. Filter and dilute to 1 liter. Standardize dye solution with known standard and titrate the prepared meat suspension with the standardized dye.

TABLE 1

Mean panel score values and standard errors of ham slices for saltiness, juiciness, tenderness, flavor, uniformity of color and color stability for various treatments

	Ascorbate		Ascorbate-Phosphate		Cal. Lactate	Commercial	Control
	Hot	Chilled	Hot	Chilled	Hot		
Saltiness	6.34	5.98	6.43	6.56	6.47	5.76	6.06
S. E.	0.28	0.79	0.35	0.80	0.42	0.91	0.38
Juiciness	6.05	6.35	6.28	5.98	5.97	5.57	5.59
S. E.	0.45	0.44	0.61	0.53	0.38	0.95	0.73
Tenderness	7.02*	6.97*	6.27	6.24	6.07	6.35	6.35
S. E.	0.36	0.28	0.56	0.46	0.32	0.71	0.49
Flavor	6.32	6.32	6.31	6.38	5.93	5.70	5.99
S. E.	0.51	0.29	0.38	0.46	0.39	0.65	0.76
Uniformity	5.13	4.71	5.58	5.03	5.30	7.00**	5.51
S. E.	1.07	0.74	0.47	0.67	0.66	0.92	0.92
Stability (24 hrs.)	3.33	2.97	3.24	3.53	3.10	3.26	3.25
S. E.	0.58	0.62	0.51	0.74	0.17	1.38	0.63
Stability (48 hrs.)	2.92	2.37	2.27	2.38	2.30	2.31	2.94
S. E.	0.69	0.35	0.30	0.49	0.22	1.68	0.68

* Significant at .05% level. ** Significant at .01% level.

ficial in preventing discoloration when added to the curing solutions because ascorbic acid and sodium ascorbate are both completely oxidized in the curing and smoking process. This is not true in sausage material since ascorbic acid or sodium ascorbate has been found in these finished products and has been shown to aid in maintaining a desirable color *(16)*.

Organolpetic characteristics. Average scores for each treatment on saltiness, flavor, juiciness and tenderness are shown in Figure 2. All treatments were remarkably uniform as scored by the panel. In every case the treated hams ranked equal to or above the control hams as well as the commercial hams.

Saltiness. Treated ham slices were scored from moderately desirable to desirable on saltiness. Commercial ham slices were scored slightly desirable on saltiness. This was a lower score than the control ham slices received. The commercial hams were visibly dryer than either the control or treated hams. This could have resulted in a saltier taste under the method of cookery. In many instances notations on the panel score sheets indicated the commercial hams to be too salty.

Flavor. The panel gave hams receiving ascorbate and/or phosphate in the cure the same score on flavor. All of these ham slices were scored as moderately desirable to desirable as shown in Table 1 and Figure 2. Commercial hams again received the lowest score which indicated the desirability of the hams injected hot and chilled with the various additives incorporated with the cure was better in flavor than hams presently found on the market.

Juiciness. All of the treated hams were scored moderately desirable on juiciness whereas the commercial and control hams were scored slightly desirable to moderately desirable. Treated hams seemed to have more free moisture on the cut surface than either the control or commercial cured ham. This was reflected in the scores on juiciness after the ham slices were cooked.

Tenderness. Both the hot and chilled hams cured with sodium ascorbate incorporated in the curing mix-

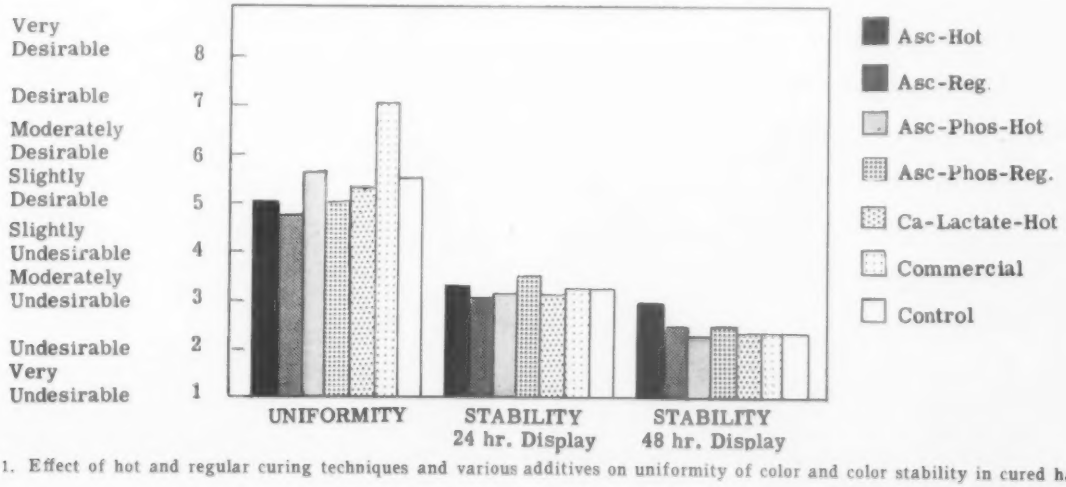

Figure 1. Effect of hot and regular curing techniques and various additives on uniformity of color and color stability in cured hams.

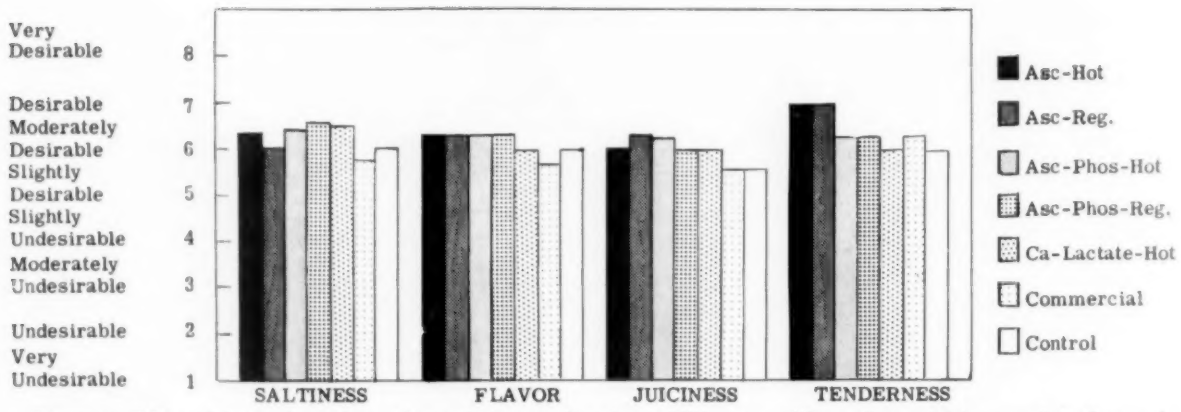

Figure 2. Effect of hot and regular curing techniques and various additives on saltiness, flavor, juiciness, and tenderness in curing hams.

ture were ranked as the most tender. Each treatment had an average score of 7.0 (desirable). The other ham slices were fairly uniform in tenderness as shown in Figure 2. All other ham slices were scored moderately desirable to slightly desirable.

SUMMARY

The method used for calculating ham weight proved to be very satisfactory since the calculated ham weight was 14.26 pounds as compared to 14.28 pounds. Standard deviation of the means were 0.64 and 0.72, respectively.

Adding sodium ascorbate and/or sodium hexametaphosphate or calcium lactate-lactic acid mixture did not result in any marked improvement in cure penetration or uniformity in color as judged by the University of Missouri meat section staff. The outer periphery of the *semimembranosus* and *semitendinosus* muscles was generally lighter in color than the remaining portion of the center ham slice.

Hams injected while hot were slightly more uniform in color than paired hams injected after being chilled.

Sodium ascorbate and/or sodium hexametaphosphate were not beneficial in preventing discoloration when these ingredients were incorporated into the curing mixture. No residual ascorbate was present in the treated hams at the end of the curing and heating process.

Discoloration was delayed considerably when ham slices were sprayed with a 5% solution of sodium ascorbate or when dipped in a solution of sodium ascorbate-nitrite mixture.

There was no appreciable difference in palatability factors—i.e., saltiness, flavor, juiciness, or tenderness—in the treated hams as compared to the commercially cured or control hams as judged by the panel. Commercially cured hams and control hams ranked slightly lower on all the palatability factors except tenderness, but there was not enough difference between any of the treatments to be significant statistically.

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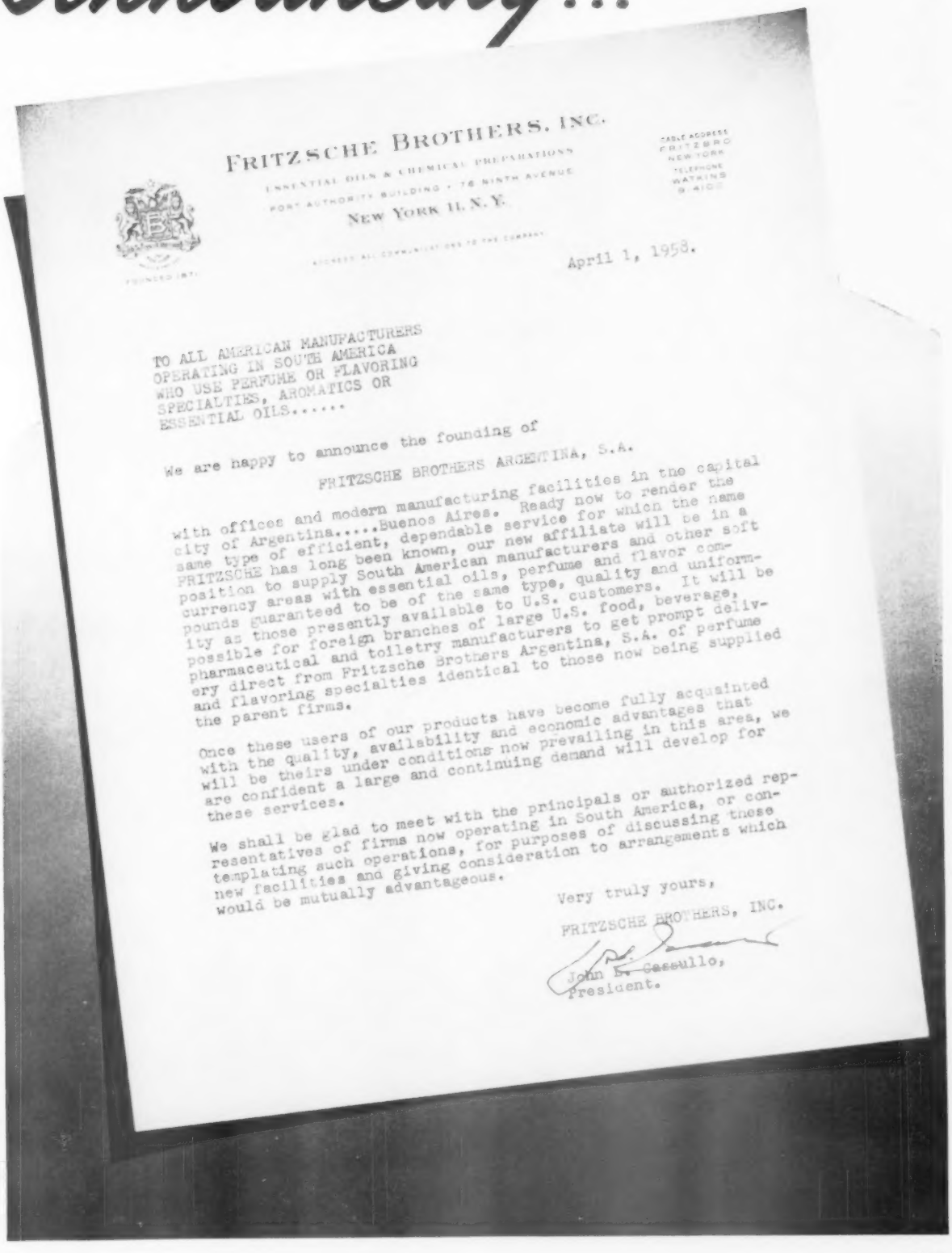

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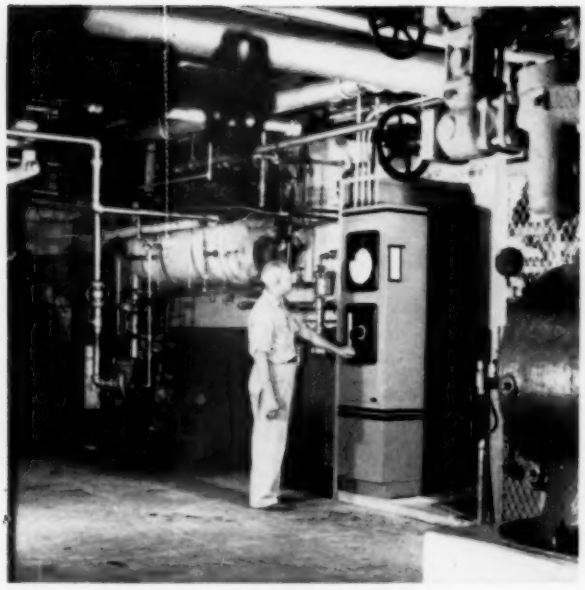

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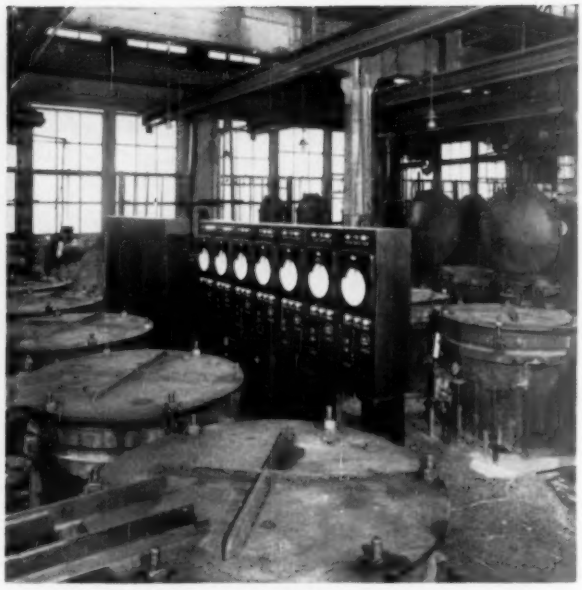

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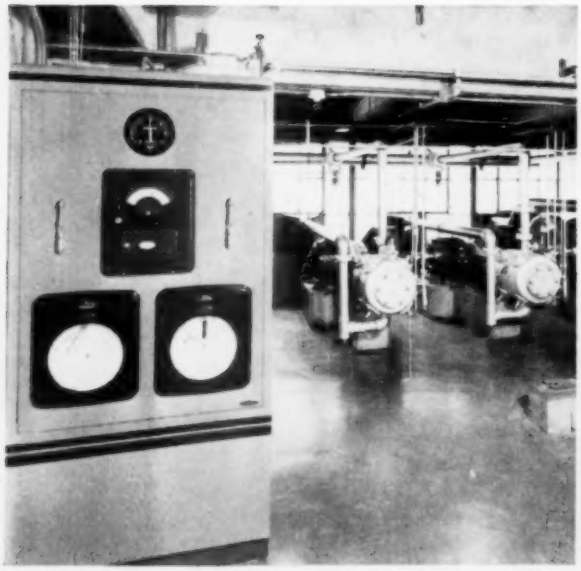

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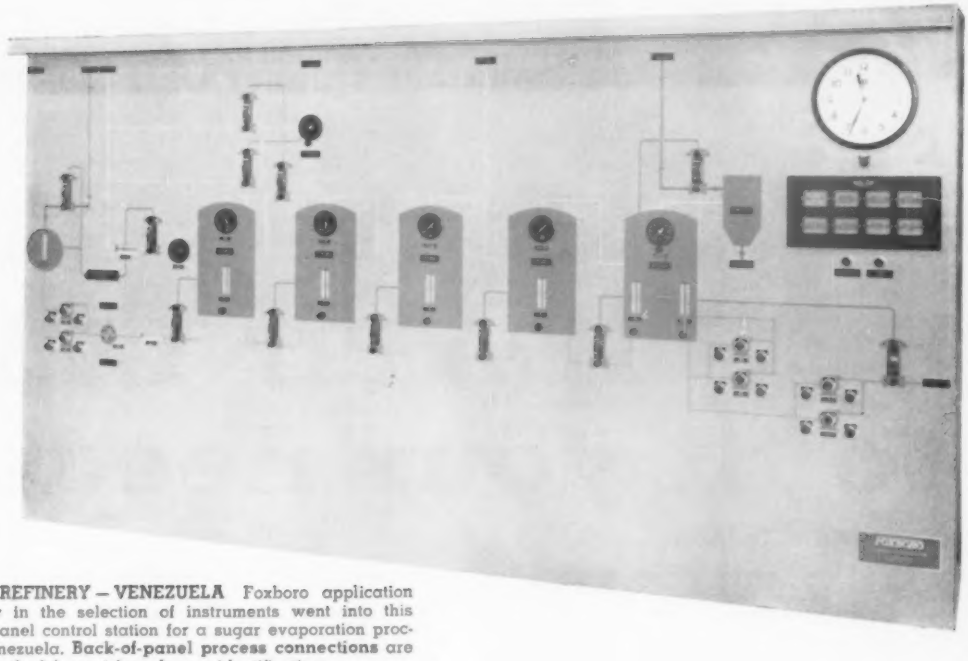

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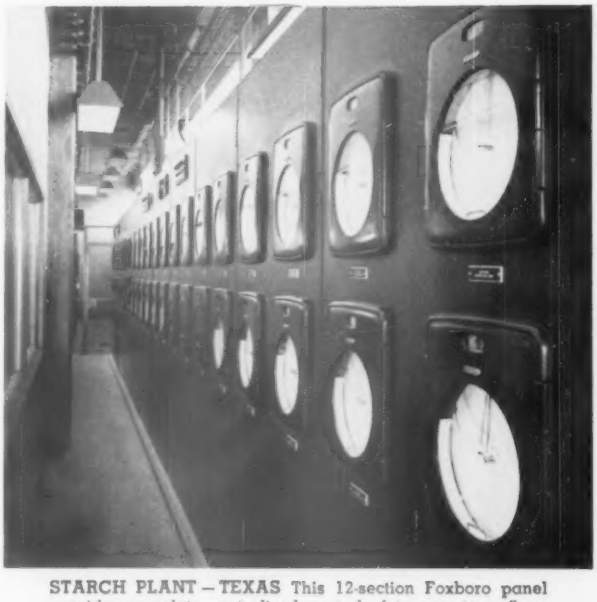

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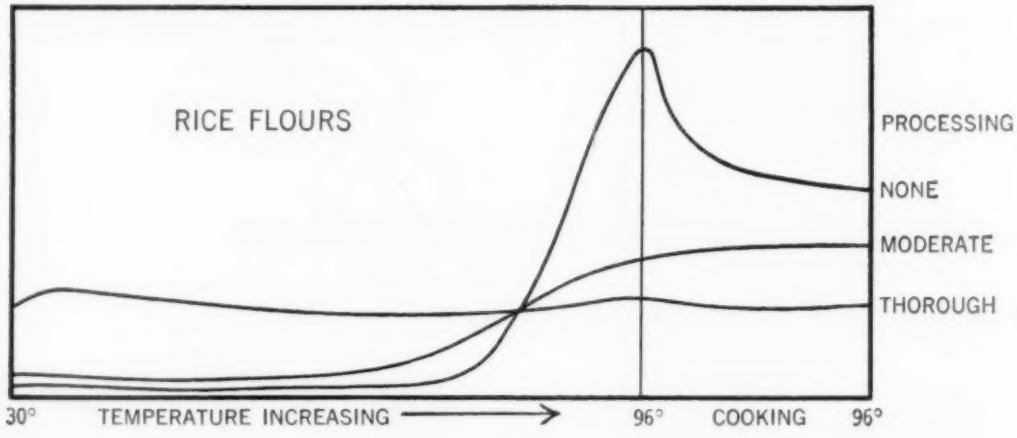

EXAMPLE: The above amylograms illustrate the effect of varying degrees of thermal treatment upon the cooking characteristics of rice flour. Raw rice flour thickens rapidly but is followed by appreciable "Set-back" upon extended cooking. Thoroughly processed rice flour hydrates well in cold water and maintains its viscosity throughout the heating and cooking cycle. Quaker can supply rice flours with any degree of processing within these limits.

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Sensory Difference Tests*

(Manuscript received September 14, 1957)

David R. Peryam

Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

THE PURPOSE of all testing is to establish differences. Since this paper does not propose to cover all sensory testing, and since boundaries are needed, it will be concerned mainly with certain methods which are commonly accepted as difference tests. First, however, let us consider some general characteristics of difference tests and attempt to establish a frame of reference for understanding the particular methods.

Characteristics of difference tests. Difference testing usually means that we are interested in "difference as such," i.e., any kind of difference, without regard to its nature or direction. For example, in the flavor quality control of a product, if strict identity with the product standard is desired, one uses a difference test; but if all that is wanted is assurance that each production lot is at least as good as the standard, some other approach, such as preference, is used. Results of difference tests are easily interpreted—either the panel discriminated or it failed to discriminate. A related characteristic is that they are usually based on statistical models having a known theoretical distribution of responses that would result from chance if the panel were completely unable to discriminate. A third characteristic is that the subject's task is completely defined in the experimental situation, so that all he has to bring to the test is his sensory capacity, plus some native intelligence. He does not have to remember standards or bring his past experiences to bear, but needs only to compare and contrast the stimuli presented. But this "general principle" is often violated—and to good advantage. A fourth characteristic is that the range of the responses allowed is usually quite limited. A test unit is set up so that the subject makes a single choice which is either "correct" or "incorrect." It is "correct" if it supports the hypothesis that a difference exists, and "incorrect" if it does not. In reviewing the test methods these "essential" characteristics will serve as reference points.

Statistics. It would mean little for just one or two people to choose correctly upon one or two occasions. It could easily happen by chance. But how many people, on how many occasions, must be correct before we may infer that we have a real difference? Here statistical interpretation is needed as a means of correcting for chance. The statistical model is a simple one and several methods of analysis are possible. The t or binomial distributions are perhaps the most often used; however, all methods give similar results. Charts or tables showing the significance of results can be easily constructed *(8)*. Since the statistical analysis has been adequately covered elsewhere *(12)*, it will not be treated in detail here; however, two important factors determining the significance of a test result should be noted. As the difference between the obtained percentage of correct responses and the chance percentage increases, and as the number of responses increases, the probability that the result could have happened by chance decreases.

TEST METHODS

Figure 1 shows the triangle test. This is the best-known form, most used and most written about, and perhaps also the most useful. The method was first published in 1946 by two

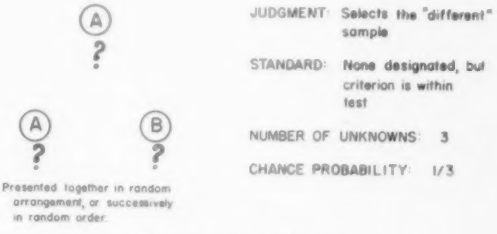

Figure 1. Triangle test.

Danish statisticians, Helm and Trolle, who described it in connection with flavor quality control work for a brewery *(9)*. It was developed independently by an American distiller in 1941 for use in research and control *(16)* and probably was used still earlier by others.

Here a test, or test-unit, consists of 3 samples. Two materials, *A and B*, are tested to determine if they differ. The subject is given 3 samples, two *A's* and a *B*. All three samples are coded, so that as far as the subject is concerned they are "unknowns." However, he knows that there are two of one and one of another, and he is told to pick the "different" sample. Thus, there is no designated standard, but the criterion for judgment is included in the test, i.e., it can be developed on the basis of these 3 samples alone. Either of the two test materials may be duplicated, and sometimes the practice is followed of alternating the material to be duplicated from one subject to the next. Usually the samples are presented simultaneously in random arrangement, although they can be presented successively in random order. Finally, let us note that since there are three unknowns, chance probability is one-third, i.e., on the average one response out of three would be correct even if the materials were identical.

The paired test (Figure 2) lacks one of the general characteristics of difference tests listed above. The standard is not immediately present, but is a subjective, predesignated one which the subject brings to the test situation. The subject is given 2 samples, again either successively or simultaneously, and is told to select one of the two according to the given criterion. To make this test effective the criterion must be such that it is understood and reacted to by all of the subjects in the same way, e.g., saltiness, sweetness, spoiled flavor, hardness, or some other familiar property. If not, the test becomes merely a mat-

* This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 780 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

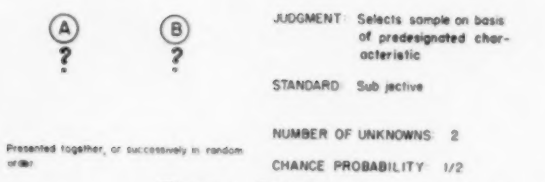

Figure 2. Paired test.

tér of voting, and one cannot interpret a negative result to mean that the panel failed to detect a difference but only that they failed to agree. Since there are 2 unknowns, the chance probability of a correct answer is one-half. This test is simply a special application of the psychophysical method of paired comparisons which has been standard for more than 75 years *(7)*.

Figure 3 represents another paired test, but with the standard present. This is the duo-trio test, originally developed by the Joseph E. Seagram Quality Laboratory in 1941 *(16)*. One of the paired samples is identified and presented first. Then the subject gets another *A* and a *B* as unknowns, in random order. The time interval between samples can be varied as desired. The subject's task is to pick the "different" sample. Again, as in the triangle, the standard is back in the test situation, and we even show the subject what to look for. Since we have reduced the unknowns to two, the chance probability of being correct is one-half, rather than one-third as in the triangle.

Figure 4 is another variant of the paired test, which has been given the name, "dual standard." Here both *A* and *B* are presented first as knowns, then they are given as unknowns and the subject has to identify them. This test seems easier than the others because the subject is given a complete "dry run" and one might expect a greater degree of success. However, there are disadvantages, particularly in taste tests where tasting the additional samples seems to be more confusing than helpful. For odor testing it does appear to give better discrimination. This procedure was also developed by the Seagram Quality Laboratory *(16)*.

A new kind of situation is represented in Figure 5. If we can use two *A*'s, what is to prevent us from using three, or even more? Nothing, except that there would be no advantage when there are only two materials, and it would increase the complexity of the test. But if the standard is not completely uniform, this multiple standards test can be used. Assume that we want to test the unknown, *B*, for difference against the nonhomogeneous standard, *A*. Of course, we could test *B* individually against A_1, A_2, etc., but since we already know that the *A*'s themselves differ significantly, the results would not be very meaningful. In the multiple standards situation *B* is presented along with a series of *A*'s, with the instruction not just to pick the "different sample" but to try to find the one which is "most different," i.e., does not belong to the family. No standard is designated as such, but the criterion for judgment can be established from the test samples. Since all samples are unknown, the chance probability of success is one divided by the number of samples in the test. This method was developed at the Quartermaster Food and Container Institute for the Armed Forces, Chicago, for use with odor stimuli. It is not well suited for taste testing because of the number of samples involved.

Figure 6 shows another less-well-known form. Here we are concerned not with a single judgment, but with a whole series. Two names are applicable—"single stimuli" because of the method of presentation, and "*A*-not *A*" because that name

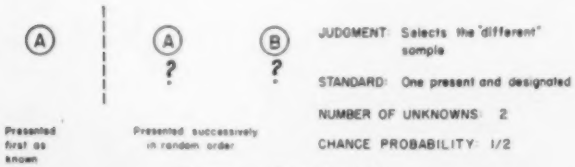

Figure 3. Duo-trio test.

was applied at Brown University where the method was studied extensively *(17)*. *A*, the standard, is first presented to the subject several times until he feels he can recognize its flavor. Then the subject is given a series of samples, any one of which may be either *A* or *B* ("not-*A*"), and he must determine which it is. An alternate approach is to present both *A* and *B* first as knowns and then to continue in the same way. The time interval between samples should be somewhat longer than for the other tests—40–60 seconds. A randomization method is used to determine for each presentation whether the sample shall be *A* or *B*. This means that the number of *A*'s and *B*'s presented in a given series may not be equal, although the proportion is a function of a random variable with a ratio of 50-50. Furthermore, the subject must be aware that the order and proportion are determined randomly. Here the standard is of particular interest. The test starts out with the standard or standards physically present and designated, but since there

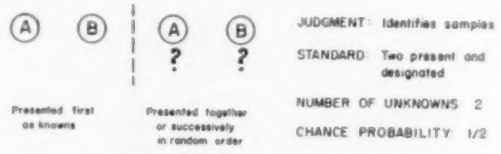

Figure 4. Dual standard test.

is no checking back, a memory criterion must take over. In long series of samples the criterion may be reinforced by presenting *A*, as a known, periodically.

Note that this test is more efficient than any of the others from the standpoint of number of responses for a given amount of tasting. A judgment is obtained for each sample instead of for each set of two or three. The chance probability of a correct response is one-half, but now the subject is selecting one of two possible answers, rather than one of 2 samples.

Figure 7 presents another multiple judgment method which is considerably more complex than the "*A*-not *A*." Here the subject is presented with a number of *A*'s and an equal number of *B*'s, all unknowns, and his task is to sort them into the two classes. No standard is designated. Figure 7 shows 8 samples, but fewer or more samples may be used. Obtaining the

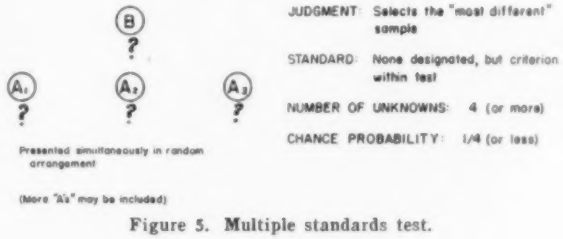

Figure 5. Multiple standards test.

chance probability is not as simple as for the other tests we have discussed. It can be shown that, for 8 samples as shown, the chance probability of a perfect solution is one-thirty-fifth, with higher probabilities for various partially correct sorts *(6)*.

Although variations of these test methods are encountered from time to time, they are of only minor importance and do not merit separate discussion because of their close similarity to the seven already described. Understanding and comparison of these methods will be facilitated by noting ways in which they may vary, as follows:

1. *Standards.* The standard may be (a) physically present and designated, as in the duo-trio, dual standard and single stimuli, (b) physically present but not designated, as in the triangle, multiple standards, and multiple pairs, or (c) it may be a designated quality to be remembered by the subject, as in the paired test.

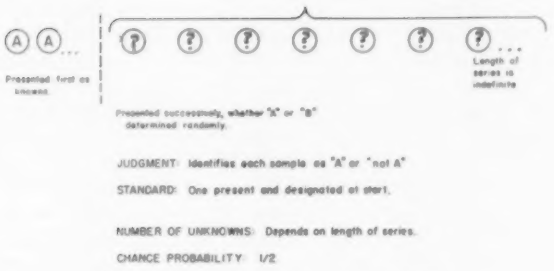
Presented first as standard
Length of series is indefinite
Presented successively, whether "A" or "B" determined randomly.
JUDGMENT: Identifies each sample as "A" or "not A"
STANDARD: One present and designated at start.
NUMBER OF UNKNOWNS: Depends on length of series.
CHANCE PROBABILITY: 1/2

Figure 6. *A*-not *A* test (single stimuli).

2. *Statistical efficiency.* As the number of unknowns in the test situation increases the expected chance proportion of correct responses decreases. This variable is often referred to as "statistical efficiency" *(12)*, the test with the lower chance level being considered the more efficient.

3. *Practical efficiency.* A more practical measure of efficiency is obtained by considering the number of samples involved as well as the chance probability of success on a given try. Some tests give much more information per sample tasted. For example, compare the triangle and single stimuli for 12 samples. With the triangle the chance of all correct responses would be $(\frac{1}{3})^4$ or $\frac{1}{81}$, since there would be four tests. With the single stimuli, allowing for two trials on the known standard, it would be $(\frac{1}{2})^{10}$ or $\frac{1}{1024}$.

4. *Psychological complexity.* The tasks represented in the various tests vary in complexity and difficulty, which, in general, increase with the number of samples which must be considered in arriving at a judgment. The more samples, the greater the possibility of adaptation and interference, and the more demands are placed on the subject's skill and attention.

TEST PROCEDURES

Nearly everything that could be said about necessary physiological and psychological controls in difference testing would be just as true about any sensory testing. However, there are a few points which are especially relevant to difference testing and should be mentioned here.

In tasting a group of samples which constitute a unit, as in the triangle test, there is competition between adaptation and memory. If the time interval between samples is lengthened to permit greater deadaptation it throws a greater burden on flavor memory. Some experimenters attempt to control the interval but more often it is left up to the subject, particularly in triangle testing when samples are presented simultaneously. Experienced panel members seem to be able to work out their own individual methods satisfactorily. Also, it is noted that they tend to allow shorter time intervals than might seem desirable to the novice. It is evident that one should not be arbitrary or dogmatic in regard to these time intervals. Each situation and type of material should be considered on its merits.

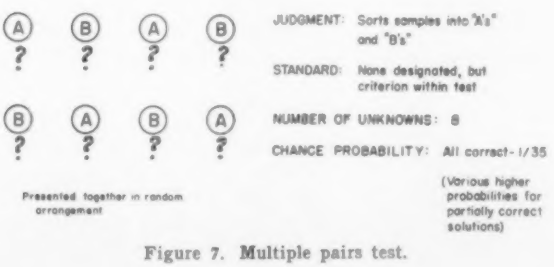
JUDGMENT: Sorts samples into "A's" and "B's"
STANDARD: None designated, but criterion within test
NUMBER OF UNKNOWNS: 8
CHANCE PROBABILITY: All correct - 1/35
(Various higher probabilities for partially correct solutions)
Presented together in random arrangement

Figure 7. Multiple pairs test.

In reviewing the test methods, single test units were described for the most part. However, it is common practice with most of the tests to give a subject more than one test unit at a session. The effect of such multiple testing on sensitivity and discrimination seems to depend on the product. Brandt *(3)* and Mitchell *(14)* have shown that there is no loss through at least 6 successive duo-trios in testing alcoholic beverages and Pfaffmann *(17)* has shown that people can test fruit juices for about 40 minutes without loss of sensitivity. However, for some products, e.g., maple syrup, adaptation takes place rather quickly and no more than one or two test units should be included in a session *(11)*.

It is well to keep in mind that the ostensible standard may not be the only one which is operative and sometimes may not even be the most important. A test subject may develop his own subjective criterion, particularly when considerable testing is done with a constant standard. When this happens in the triangle or duo-trio the third sample is not necessary, and may even be harmful. One could just as well use the simple paired test or the single stimuli. Byer and Abrams *(4)* showed that, when the subject knows the kind of difference to expect, the paired test is superior to the triangle. Raffensperger and Pilgrim *(18)* demonstrated that telling people what sort of a difference to expect improves discrimination in the triangle itself.

Subjects will tend to use all available clues in trying to solve the difference test problem. Even though certain kinds of differences will not help, a person may allow them to affect his judgment. For example, with a solid food there are apt to be many differences among the samples—texture, particle size, shape of portion, etc.—that are purely incidental and have nothing to do with the essential flavor difference which is under test. In such a case the instruction to "pick the different sample" means nothing because all the samples are different. The subject has to know what sort of differences to look for before his response will be meaningful. This is one reason why difference tests are used most often with homogeneous materials.

It is essential to eliminate all incidental differences between the samples that may be correlated with the factor being tested. Otherwise one is likely to misinterpret the results. This caution most often applies when visual differences are associated with possible flavor differences, but are unimportant from the experimenter's standpoint.

PANEL SELECTION AND TRAINING

The assumption is reasonable that it should be possible to select groups of people whose performance on sensory tests will be superior to that of the general population. Various schemes based on intuition, rational judgment, or experimentation have been tried, but with varying degrees of success *(2, 5)*. Further,

even though a theoretical system based on experimentation may be available, actual selection may be based on simple practical criteria. One of the major problems is the amount of pretesting work required to effect reliable selection. A further difficulty may be the experimenter's inability to specify accurately the nature of the panel member's task. "Quickie" methods, based upon a few tests, even complex ones, generally have not been very satisfactory. The tedious process of selecting on the basis of sensitivity to the four basic tastes is often recommended, and has even been tried *(13)*, but the method is of doubtful value. Sensitivity is only one factor; successful performance requires such skills as the ability to remember flavors and to compare flavors and flavor strengths in spite of the time lag between samples. Interest and motivation are also very important.

With difference testing, panel selection appears somewhat more feasible than with other types of tests, because an objective criterion of performance is possible. Experience in industry where difference testing is used for flavor quality control on a continuing basis *(10, 15)* has demonstrated the validity of selecting panel members. Here it is feasible because they use panels who test repeatedly, thus providing an adequate sample of each person's behavior. All panel members take essentially the same tests and individual performances are continually evaluated. Those who are right most often stay on the panel, the less skillful are dropped. Potential new members are simply started in the system and are retained only if their relative skill so merits. Thus, in effect, future performance is predicted from past performance.

Although it is commonly assumed that a person needs some training, there is little information available which bears specifically on this point. Industrial experience in flavor quality control work seems to show that training is helpful *(10)*; however, the improvement in over-all panel performance which is usually noted may be due to the selection process rather than to training as such. Another possibility is that a subject's performance will improve because he becomes more familiar with the product being tested, but not because he develops a general skill. Raffensperger and Pilgrim *(18)* found that subjects with no prior experience in difference testing discriminated as well as an experienced panel when appropriate instructions were given. Too little is known to be dogmatic about how much and what kind of training is necessary fully to qualify a panel member. While not discounting the value of having experienced people, one should be aware of the possibility that only a small amount of training may be required in a given instance.

INTERPRETATION

The question is often asked, "How many panel members are needed?" One aspect of this problem is concerned with the reliability of the result. A sufficient number of responses is needed to assure that an important difference will be proved statistically, but this problem is easily solved. One can determine mathematically the number of judgments required to reach any desired level of significance with any given degree of discrimination as shown by the percentage of correct responses. However, the number of judgments can be increased either by using more people or by getting more judgments per person, and this points to the other facet of the problem. The two approaches are not statistically equivalent. As the number of people on the panel is reduced, the generality of the test result is progressively restricted. Perhaps this is of no great concern since the usual difference test panel could hardly represent any definite population anyway. It may be assumed that the population of real interest, about whose behavior we want to make inferences, is that composed of the potential consumers of the product. It is further assumed that the difference test panel is more discriminating than the general consumer—how much more we do not know. Selection and training of panel members and the development of optimum test procedures are designed to assure this; and one can be reasonably certain that a difference not readily apparent to the panel will never be detected by the consumer. This leaves a rather broad range for exercise of the experimenter's judgment; however, in practice, panels are generally small. Panels of less than 5 persons are seldom used, and panels of more than 20 are also rare. Decisions are seldom based on fewer than 16 judgments and it is unusual to obtain more than 30.

Difference test results are limited by the fact that they show only that a particular group of people, at a particular time, and under the conditions of the test, were able to distinguish between two materials, or else failed to prove this within the prescribed test length. Usually we assume that the criterion of difference used by the panel members was related to some physical variable being investigated, but we cannot be sure. Nor are we sure that the difference was the same for all those who responded correctly. Unless there is independent evidence, we do not know whether one sample is better or worse than the other, more or less off-flavor, etc. The exception is the paired test, where a criterion must be specified. Otherwise, all we have is a probability that a discriminable difference exists.

Do the results give degree of difference, as well as the probability of an unknown degree of difference? The answer is "Yes." Intuition tells us that this is true, and so does rational analysis. When we are dealing with a difference in the threshold range, the only appropriate measure of degree of a difference is the frequency with which it is noticed. Thus, an increase in the percentage of correct responses can legitimately be interpreted as indicating greater difference. Note, however, that greater statistical significance resulting from a larger number of responses, with no change in per cent correct, cannot be so interpreted.

APPLICATIONS

One important function is that of controlling the flavor quality of a product against a pre-selected standard. The best examples are in the alcoholic beverage industry, where they have been very effective in achieving product uniformity *(10)*. If product uni-

formity is a good thing, why don't more companies use the difference testing methods? One answer is that difference tests may impose a more rigid control than many products can afford; also in many instances there is no real need for rigid control. There can be minor variations in a product which would have no practical bearing on consumer acceptance. Here difference-as-such should give way to difference on some continuum such as preference or quality. We want to know not only whether the production lot is different from the standard, but also which is better.

The difference methods are applied extensively in laboratories that do research and development work on foods and beverages *(2)*. A complete review cannot even be attempted here, but they can be applied to such problems as the effect of formulation or processing changes, use of different raw materials, or the effect of packaging materials. The triangle is often used to establish thresholds *(1)*. This is a legitimate use, but the method is cumbersome and inefficient, and the standard psychophysical methods are more suitable.

The triangle test, in particular, has become perhaps a little too famous and has acquired an aura of scientific respectability that may mislead the uniformed. People who are unsophisticated about sensory testing may attempt to use it where it is not appropriate. A frequent error is to attempt to combine a difference test—usually the triangle—with a preference or quality judgment test. If the real problem is something more than simple difference, the information obtained from the difference test does little good. Its inclusion complicates the situation and tends to divert the subject's attention from the dimension of real interest. There is evidence, too, that it will bias the results. Schutz and Bradley have shown that when a preference judgment is asked for, following a triangle test on the same samples, subjects will tend to prefer the paired samples *(19)*.

COMPARATIVE EVALUATION

These difference testing methods are similar in many respects, but there are obvious differences. Some are functionally equivalent; others are not. Although it cannot be precise, an attempt at comparative evaluation will emphasize some important relationships.

Considering the purpose for which they are used, the primary criterion of value for difference tests should be sensitivity, i.e., that test should be considered better which will detect the smaller degree of flavor difference or will demonstrate a given degree of difference with greater certainty, or with less effort. Sensitivity *per se* does not appear to be a very useful criterion. Considerable work has been done to assess relative sensitivity; however, it has not resulted in consistent clear-cut evidence showing the relative merit of the various methods *(17)*. Discrimination, as empirically determined, is too often affected by factors other than the test method itself. Therefore, it will be more useful for our purposes to consider some of the more important subsidiary characteristics, which include efficiency, simplicity and appropriateness to the problem.

Efficiency relates to the amount of work required to achieve a given degree of discrimination. We have already noted that two aspects should be considered. Statistical efficiency is interpreted as the inverse of the chance probability of a correct response, e.g., those tests with a chance probability of $\frac{1}{2}$ would be considered equal and lowest in statistical efficiency whereas the multiple pairs test (Figure 7), with a chance probability of $\frac{1}{35}$, would be considered highest. This aspect has been given more attention than it deserves and has sometimes been erroneously represented as a measure of the sensitivity of a test. In determining practical efficiency one must consider the number of samples tasted in arriving at a judgment as well as the chance probability of a correct judgment. For example, if we equate the total number of samples at 8 or 9 (as appropriate to the test form), then determine the chance probability of all responses being correct, we get the following order of decreasing efficiency: "*A*-not *A*", multiple pairs, triangle, multiple standards and simple paired, duo-trio, and dual standard.

The efficiency of a test, thus defined, cannot be considered independently of simplicity; they interact in determining over-all sensitivity. We are entitled to assume that the simpler test is better, other things being equal. This is a good practical guide even though the superiority of the simpler method may not have been proven in each case. The less the panel member has to draw on complex skills, the less opportunity there is for error, and the less chance for loss of effectiveness due to the selection of inadequate subjects or to the flagging of attention during continued testing. The main factor in complexity is the number of samples, particularly the number of unknowns, that must be considered in arriving at a single judgment. Hence, there is a basic conflict with statistical efficiency. The 7 forms may be ranked as follows in order of decreasing complexity: multiple pairs, multiple standards, triangle, duo-trio, dual standard, single stimuli, and paired. The inversion on the last two is because of the necessity for remembering the standard in the single stimuli.

Appropriateness is a different kind of criterion. It cannot be applied generally, but is specific to the problem and test situation. The necessity of being logically appropriate may restrict the choice of method for certain problems, or a certain method may be found especially suitable for a particular problem. For example, one can not use the paired test unless the dimension and direction of difference can be clearly specified. Again, the multiple standards test is not appropriate except in the unusual situation where the standard is a family of materials rather than a single material. In selecting a test method for regular use, pertinent elements which are anticipated in the test situation should be considered, for example, the probable constancy of the panel, their level of motivation, the amount of testing to be done, whether testing is to be continuous or intermittent, and whether the materials are constant or varied.

Finally, let us try to assign the methods a relative order of merit by consensus, as indicated by their usage. The triangle must be accorded first place because of its wide variety of uses in research, development, and quality control. Next is the duo-trio because of its extensive application in flavor quality control. It could apply wherever the triangle does, although it is not as popular. The paired test deserves third place because of its many research applications, but here the question is always pertinent whether, in any particular test, one is measuring "difference as such" or difference on some particular dimension. When the dimension is a simple one, such as "sweetness," we may call the paired test a difference test; but not when the dimension is a general one, such as "preference" or "quality." The multiple pairs test is ranked fourth because of its frequent appearance in the literature, although usually the emphasis has been on the evaluation of panel members rather than on the testing of materials. It is hardly ever used for anything else. The multiple standards, dual standard, and "*A*-not *A*" tests are tied for last place, since they are seldom used. They are specialized tests and are typical of many other forms which could be developed if needed.

USE OF THE RATING SCALE

The methods described above do not exhaust all possibilities for the measurement of sensory differences. One of the most useful approaches, the rating scale, has been omitted. This basic technique can be applied with many functionally different problems, but is not specifically identified with any one of them. Therefore it can not be considered as belonging to the family of difference tests, although it is often used for difference testing and should be discussed here in order to show its relationship to the other methods.

Such use requires either a verbally designated criterion, as in the paired test, or one which is present and designated, as in the "*A*-not *A*". When using a verbal criterion the restriction again applies that it must be understood by all of the subjects in the same way. Samples are presented singly and the subject indicates the amount or intensity of the designated characteristic on a rating scale. For example, Schutz and Pilgrim *(20)* in measuring differences in sweetness among sugars used a 9-category scale with alternate points anchored as follows: *none, slight, moderate, large, extreme.* This type of judgment is similar to that required in the paired test, where each sample is compared to the other, or in the "*A*-not *A*" method where only the presence or absence of the characteristic is noted, but is more complex than either.

In another application of the rating scale a physical standard is constantly available. The subject considers each unknown in turn, comparing it against the standard and estimating the degree of difference between them. The estimate is recorded on a rating scale like that described above.

The rating scale method is not limited to comparison of only two materials at a time, as are most of the others, but as many as 4 or 5 different samples may be rated at one session. Each subject tests all samples in the group. Successive integral values are assigned to the scale points and the resulting distributions are analyzed by some appropriate technique, such as analysis of variance, to test for the significance of differences between samples.

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Chlortetracycline for Preserving Gulf Oysters

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IN A RECENT PUBLICATION by Farber *(5)* a literature review was presented which briefly discussed previous results with chlortetracycline (CTC) and other antibiotics as aids in the preservation of fish fillets and shrimp. Farber found that CTC and hydroxytetracycline had a definite preservative effect on certain fish fillets, but none for shrimp in the concentration of 2 p.p.m. Abbey, Kohler and Upham *(1)* reported on the effect of CTC in the processing and storage of freshly shucked Chesapeake Bay oysters, and their data indicated that shucked oysters treated with 1 p.p.m. to 30 p.p.m. CTC remain organoleptically acceptable for longer storage periods when compared to untreated controls. Similar findings were reported by Boyd and Tarr *(3)* in which the sour odors typical of spoiled oysters were inhibited by CTC.

Several processes for using chlortetracycline as a preservative agent for extending the storage life of refrigerated, freshly shucked Gulf oysters were explored.

EXPERIMENTAL

Chlortetracycline for preserving fresh ice stored oysters. Three experiments were designed to obtain adequate data for determining the amount of CTC required for washes or dips, prior to iced storage and the best methods for its employment. Experiments 1 and 2 were made to compare the bacterial inhibition resulting from dips containing 10 and 20 parts of CTC per million parts of washing solution. Experiment 3 was planned after it became obvious the effectiveness of CTC dip or washes in inhibiting bacteria on oysters depends extensively on the amount retained by the oysters. Since only a thin film of the solution remains on the oysters after draining from a dip, it was decided to study the use of a material which adheres to the oyster surface and which consequently might increase the amount of antibiotic retention. In this latter study, a Kelco solution containing 20 p.p.m. CTC was prepared so that it would set to a gel within 30 minutes and its preservative action was determined.

For the first test, commercially shucked select oysters purchased in New Orleans, were packed unwashed in gallon cans, placed in ice in arctic hampers, and transported to the laboratory, where they were processed within three hours. Two gallons of oysters were washed in 3 gallons of water for 3 minutes, drained in colanders 3 minutes, and distributed into pint cans. Another 2 gallons of oysters were washed in 3 gallons of 10 p.p.m. CTC solution for 3 minutes, drained in colanders 3 minutes, and distributed into pint cans. All samples, except those separated for initial analyses were packed and maintained in crushed ice in separate boxes. Determinations were made on alternate days on pH of the oyster meat and drip, amount of drip, general appearance and bacterial counts on the oyster meat.

In the second test, the procedure was a repetition of the first experiment except 20 p.p.m. CTC solution was used for the antibiotic wash, and the oysters were packed in cartons instead of cans. To better estimate the value of washing, unwashed samples were used as controls. Bacterial counts on the oyster meat were made on the samples in this series. Trimethylamine nitrogen analyses *(2, 4)* were made to determine if this test warranted investigation as a possible method for measuring the relative quality of ice stored oysters.

In the third test, extra select, freshly shucked unwashed oysters purchased from a packing house in New Orleans were packed in ice and transported to the laboratory in Baton Rouge, where they were processed as follows within 4 hours after shucking: (1) control: 6 pints of oysters were washed in 6 pints of water for 3 minutes, drained 2 minutes in a colander and put in jars; (2) CTC dip: 6 pints of oysters were washed in 6 pints of water containing 20 p.p.m. CTC for 3 minutes, drained 2 minutes and put in jars; (3) CTC-Kelco gel dip: 6 pints of oysters were washed in 6 pints of water 3 minutes, drained 2 minutes, then dipped in CTC-Kelco gel for 3 minutes, drained 2 minutes and put in jars. All oysters were well coated with the CTC-Kelco gel; (4) CTC-Kelco gel: 6 pints of oysters were washed in 6 pints of water for 3 minutes, drained 2 minutes then put in jars containing CTC-Kelco gel (about 1 inch of gel per jar). The oysters did not sink into the gel, so the jars were sealed and rotated. All oysters became coated with gel.

The Kelco gel was prepared as follows:

Kelco gel	55.0	g.
Calcium tartrate	10.0	g.
Citric acid	25.0	g.
CTC	0.0964	g.
Water	1.25	l.

All of the jars were packed and maintained in crushed ice. The following observations and test were made every third day: general appearance, odor, color, loss on cooking, organoleptic score, and bacterial counts on the oyster meat.

Organoleptic scores for experiments reported in this paper were obtained by the following procedure: weighed oysters were placed on screens in small aluminum pans fitted with lids, and containing one tablespoonful of water. The covered pans were placed in preheated ovens and steam cooked at 400° F. for exactly 15 minutes, after which time the oysters were removed, cooled and reweighed. Loss of weight on cooking was calculated from these figures. Taste tests were made on the basis of flavor (40%), juiciness (20%), texture (20%) and saltiness (20%). A value of 100 represents a maximum organoleptic score.

RESULTS

Results from these three tests are presented in Tables 1, 2 and 3.

Chlortetracycline for preserving frozen oysters. In this experiment the original appearance of each oyster was recorded for the purpose of comparison in determining the value of CTC for preventing spot formation on oyster bodies. Select oysters were separated into two groups (1) those with no spots or faint spots, and (2) those exhibiting pronounced spots. The percentage of each type was recorded. After making the above separation, the oysters were washed in water for five minutes, drained two minutes, and each of four portions treated as follows: (1) controls: packaged in polyethylene bags, heat

TABLE 1

Comparison of water wash and CTC wash for oysters prior to ice storage

Days	Water washed			C.T.C. 10 p.p.m. washed		
	pH		Bacterial count x 10^6 (Oyster meat)	pH		Bacterial count x 10^6 (Oyster meat)
	Liquor	Meat		Liquor	Meat	
0	6.72	6.10	0.28	6.88	6.08	0.32
2	6.55	5.99	0.27	6.50	5.99	0.28
4	6.42	5.98	0.25	6.40	5.95	0.24
6	6.36	5.77	0.51	6.30	5.77	0.42
8	6.18	5.82	0.98	6.08	5.80	0.67
10	6.00	5.70	1.34	6.03	5.75	0.65
13	5.85	5.68	2.11	5.87	5.70	0.96
15	5.75	5.60	3.78	5.72	5.62	1.26
17	5.70	5.59	6.86	5.72	5.59	3.10
19	5.63	5.53	13.90	5.69	5.59	9.64
21	5.62	5.47	30.40	5.62	5.50	20.10

sealed and stored at 0° F.; (2) packaged in polyethylene bags, frozen without sealing, then glazed with 20 p.p.m. CTC, heat sealed and stored at 0° F.; (3) 20 p.p.m. CTC washed for five minutes, drained two minutes, packaged in polyethylene bags, heat sealed and stored at 0° F.; and (4) dipped into 20 p.p.m. CTC in Kelco gel (pH 6.5–7.0) for five minutes, drained two minutes, packaged in polyethylene bags, heat sealed and stored at 0° F.

Data on unfrozen samples and tests made monthly included odor, general appearance, % no spots, % pronounced spots, % dark edges of mantle, loss in weight upon cooking, and organoleptic score.

RESULTS

Results on the % spots and intensity, and the % dark edges of mantle are given in Table 4. Loss of weight on cooking and organoleptic scores are presented in Table 5.

DISCUSSION

CTC treated oystsers stored in ice had lower bacterial counts (Tables 1, 2 and 3), after approximately 3 weeks than untreated controls. The odor and appearance of the treated oysters used in the first experiment (Table 1) were notably superior even though the pH values were similar. This result supports the evidence of Abbey *(1)* that pH trends appeared to be less indicative than bacterial counts and organoleptic studies as useful quality guides for oysters processed with CTC. Twenty p.p.m. CTC were more effective than 10 p.p.m. for bacterial inhibition. Oysters with low initial counts are better protected by CTC than oysters with high initial counts where some decomposition already may have occurred because of their presence.

Trimethylamine nitrogen values indicate a possible application of this test, but more extensive experiments are required before the method can be evaluated as a quality test for oystsers (Table 2).

The use of Kelco gel (Table 3) for the purpose of increasing retention of CTC on the oyster surface was unsuccessful. Oysters treated with CTC-Kelco gel dip and those packed in CTC-Kelco gel were organo-

TABLE 2

Bacterial counts and trimethylamine n. of meats of oysters unwashed, washed in water, and washed in CTC solution prior to ice storage

Days	Unwashed		Water washed		C.T.C. 20 p.p.m. washed	
	Bacterial count x 10^6	Trimethylamine mg. N/ 100 g.	Bacterial count x 10^6	Trimethylamine mg. N/ 100 g.	Bacterial count x 10^6	Trimethylamine mg. N/ 100 g.
0	0.04	0.00	0.05	0.00	0.04	0.00
2	.....	.....	0.08	0.15	0.05	0.19
4	0.12	0.09	0.14	0.09	0.04	0.04
6	.....	.....	0.27	0.28	0.06	0.38
8	0.39	0.24	0.44	0.22	0.10	0.16
10	.....	.....	.....	0.32	.....	0.20
13	19.40	2.30	2.32	2.10	.....	2.05
15	.....	.....	.....	2.40	0.80	2.40
17	26.20	3.40	.....	3.10	0.60	2.40
20	Sample completely spoiled	Sample completely spoiled	18.60	.....	0.75	.....

TABLE 3

Effect of CTC and Kelco Gel on bacterial counts, cooking loss and organoleptic scores of ice stored gulf oysters

Age in days	Control			20 p.p.m. CTC dip			20 p.p.m. CTC-Kelco gel dip			20 p.p.m. CTC-Kelco gel		
	Bacterial count x 10^6 (Oyster meat)	% Loss on cooking	Organoleptic score	Bacterial count x 10^6 (Oyster meat)	% Loss on cooking	Organoleptic score	Bacterial count x 10^6 (Oyster meat)	% Loss on cooking	Organoleptic score	Bacterial count x 10^6 (Oyster meat)	% Loss on cooking	Organoleptic score
0	.032	57.3	96.0	.032	57.3	96.0	.032	57.3	96.0	.033	57.3	96.0
3	.106	60.8	96.7	.033	57.8	93.3	.033	63.5	93.3	.022	62.8	86.7
6	.328	58.7	76.7	.069	59.6	86.7	.057	64.4	76.7	.051	68.9	66.7
9	.421	58.5	80.0	.095	60.8	76.6	.062	63.2	60.0	.059	64.3	56.7
12	.497	59.4	73.3	.277	62.2	56.7	.084	66.1	53.3	.100	71.0	50.0
15	.600	60.8	63.3	.404	59.7	70.0	.093	spoiled	spoiled	.092	spoiled	spoiled
18	.815	66.2	60.0	.652	62.5	60.0						

TABLE 4

Effect of CTC and Kelco Gel on percent spot formation in frozen gulf oysters stored at 0° F.

Age in months	Control			20 p.p.m. CTC glaze			20 p.p.m. CTC wash			20 p.p.m. CTC-Kelco gel dip		
	No spots	Severe spots	Dark edges	No spots	Severe spots	Dark edges	No spots	Severe spots	Dark edges	No spots	Severe spots	Dark edges
0	90	10	30	90	10	30	90	10	30	90	10	30
1	88	12	52	79	21	45	83	17	42	92	8	43
2	85	15	48	71	29	47	79	26	40	89	11	44
3	56	44	52	72	28	72	67	33	64	68	32	50
4	70	30	46	76	24	56	77	23	61	76	24	90
5	64	36	84	80	20	96	76	24	72	51	50	75
6	69	39	81	83	17	48	81	19	65	72	28	71
7	63	37	88	85	15	78	78	23	92	75	25	88

TABLE 5

Effect of CTC and Kelco Gel on cooking loss and organoleptic scores of frozen gulf oysters stored at 0° F.

Age in months	Control		20 p.p.m. CTC glaze		20 p.p.m. CTC wash		20 p.p.m. CTC-Kelco gel dip	
	% Loss on cooking	Organoleptic score	% Loss on cooking	Organoleptic score	% Loss on cooking	Organoleptic score	% Loss on cooking	Organoleptic score
0	49.3	91	49.3	91	49.3	91	49.3	91
1	50.6	96	54.9	93	55.0	94	53.9	89
2	54.7	78	53.2	77	54.7	77	52.8	77
3	52.7	70	52.3	77	53.4	65	53.8	68
4	54.2	63	56.1	67	53.1	67	55.1	66
5	56.2	67	53.7	71	54.5	63	54.6	67
6	56.7	66	51.0	75	57.4	63	56.0	66
7	55.5	65	57.9	64	60.1	67	58.1	66

leptically unacceptable after two weeks. Bacterial counts were significantly lower than the control, but the oysters developed a distinct sour taste and odor which was attributed to the acidity of the gel. It is possible that less acid adjustment of pH prior to dipping or packing in CTC-Kelco gel mixes may prevent the formation of these undesirable qualities.

Even though the organoleptic scores of the control and 20 p.p.m. CTC dip were similar, consumer acceptability was improved with the CTC treated oysters because they exhibited a better appearance. A purchaser would undoubtedly prefer to purchase the treated oysters when comparing jars of treated and untreated products after two weeks ice storage.

Disregarding the oysters treated with Kelco gel, all of the CTC washed oysters from each experiment were of better general appearance and odor and possessed less off colors than the untreated oysters, although on numerous occasions this odor decreased in intensity after prolonged exposure to air. CTC did not influence the loss in weight obtained upon cooking, or the general appearance of the cooked oysters.

CTC treatment also resulted in improving the acceptability of frozen Gulf oysters during a 6-month storage period, even though no significant improvement in organoleptic scores was evident. Formation of severe dark spots was less in oysters treated with CTC glaze, CTC wash and CTC-Kelco gel dip, than those in the untreated control. CTC glazed and CTC washed oysters had the best odor and appearance after 6 months. Numerous off colors developed in the untreated controls after the first two months, and gave the liquor and some oysters a multicolored rainbow appearance. Colors developing included red, violet, brown, tan, yellow, green and orange. This tendency created a lack of consumer appeal.

CONCLUSIONS

It can be concluded from these experiments that the use of 20 p.p.m. CTC has merit as a preserving agent that outweigh cost for processing Gulf Oysters prior to iced or frozen storage. No disagreeable qualities appeared to develop because of inclusion of CTC. Under these circumstances the employment of CTC might be considered beneficial as it maintains consumer acceptance of the product by preventing the development of undesirable physical qualities, and is effective in such low concentrations.

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Protection of Cooked Meats with Phosphates

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HEAT TREATMENTS are widely applied as methods of preparing and preserving meats. Conventional canning procedures use them as the sole method of preservation whereas such newer innovations as irradiation or freezing of prepared meat products may employ only a slight preliminary heating prior to other treatments. As a result of these heat treatments, problems are encountered during the cooking and storing of uncured meat which greatly affect its acceptability. Changes in flavor and odor are the most important factors contributing to loss of palatability. Loss of juice and protein denaturation may contribute to undesirable texture. These problems may appear to be, at first thought, most prominent in meats preserved for long periods of time. However, they are encountered frequently in holding left-over cooked meats in the refrigerator for short storage periods.

The flavor of cooked meats changes rapidly during storage. Various terms such as "warmed over," "stale," or "rancid" have been used to describe this characteristic flavor. The rapidity of this change was called to the attention of the authors by Bessert *(2)*, who reported that certain cooked meats which received high palatability ratings immediately after cooking deteriorated in flavor rapidly after a few hours of refrigerator storage. When tested for oxidative rancidity by the 2-thiobarbituric acid (TBA) reagent, this loss of flavor paralleled an increase in TBA values.

If the flavor change in cooked, stored meats is oxidative in nature, the use of an antioxidant may offer a possible solution to the problem. Such an antioxidant must be effective after heating and must be accepted for use by the Meat Inspection Branch of the USDA. Phosphate additives meet these two criteria. Chang and Watts *(4)* reported that polyphosphates were effective antioxidants in artificial aqueous fat systems within the pH range of normal meat.

In addition to their antioxidant properties, phosphates have been reported to decrease loss of juice and other water soluble constituents. Bendall *(1)* reported that orthophosphates increased the swelling of lean meat which he suggested is due to increased ionic strength. He further observed that pyrophosphates produced "specific swelling" and postulated that this effect possibly involves splitting of the actinomyosin. Swift and Ellis *(12)* also demonstrated that the effectiveness of pyrophosphates is primarily due to the ionic strength and pH of the solution, but were not able to confirm Bendall's observation that pyrophosphates exert an effect superior to that of orthophosphates. Morse *(10)* has reviewed some of the findings of German investigators on imbibition of meats as affected by phosphates.

At the present time, the application of phosphates to improve the quality of meat is limited to cured products *(15)*. Two patents *(3, 5)* are currently in active use. One of these *(5)* suggests that undesirable changes in color are inhibited in cured meats by polyphosphates; the other *(3)* maintains that orthophosphates cause a reduction in shrinkage which is attributed to a change in pH.

Phosphates, although effective antioxidants by themselves, are even more effective when used in combination with ascorbic acid *(9)*. This synergistic effect has been observed in aqueous lard systems. However, in the absence of suitable synergists, ascorbic acid becomes a potent prooxidant under the same conditions *(11)*. In addition to its antioxidant properties, ascorbic acid protects the color of cured meat and refrigerated fresh meat *(17)*. Its use in the preparation of cooked, cured comminuted meat products has been approved by the USDA Meat Inspection Branch *(16)*.

Although MIB has accepted the use of phosphate additives in cured products only, their addition to uncured meats seemed highly feasible. Therefore, the studies reported herein were designed to study (a) flavor changes and fat rancidity during cooking and storing of uncured meat, (b) the use of phosphates to inhibit both juice loss and oxidative changes in pork, and (c) the synergism of phosphates with ascorbic acid.

METHODS AND MATERIALS

Sources of meats. Products used in this study were fresh meats which were purchased locally, the pork hams being obtained from a slaughtering house and the leg of lamb, beef round, veal round and chicken thighs from a super market. Previous histories of the meats were unknown. All meats were assayed as soon as possible after purchasing. They were held in refrigerator storage until the time of assay.

Preparation. The meat was ground once, using the attachment for the Kitchen-Aid mixer, Model 3-C. After thoroughly mixing by hand in order to obtain a homogeneous product, 500 g. of meat were packed tightly into No. 2 enameled cans, sealed immediately, and cooked in a steam bath to an internal temperature of 70° C. Before selecting the internal cooking temperature a range of temperatures varying from 60° C. to 95° C. was investigated. An intermediate one of 70° C. was selected, although it is realized that this temperature is insufficient for pork. After cooking, the cans were opened immediately, reground, thoroughly mixed, and stored at refrigerator temperatures (5–7° C.) for subsequent assays.

Addition of phosphates and ascorbic acid. Four types of phosphate salts were added to the ground meat at a concentration of 0.5% which is in accordance with present government regulations *(15)*. These salts were as follows: (a) sodium orthophosphate, Na_2HPO_4 and NaH_2PO_4; (b) sodium pyrophosphate, $Na_2H_2P_2O_7$ and $Na_4P_2O_7$; (c) sodium tripolyphosphate, $Na_5P_3O_{10}$; and (d) sodium hexametaphosphate, $Na_6P_6O_{18}$. All salts were added to the raw meat in the dry state. Where 2 salts were added, the two were thoroughly blended before mixing with the meat.

Before adding the ortho- and pyrophosphates a 0.5% aqueous solution of the more acid salt was prepared and then adjusted

to pH 6.4 with the basic salt. The ratio needed for such an adjustment was used in the meat. This was done in an effort to rule out pH as a factor which might affect palatability changes. No pH adjustments were made with tripoly- and hexametaphosphates. In general, the tripoly treated samples were higher in pH and the hexameta slightly lower than the controls. Accurate adjustment of pH is difficult, especially in cooked meats, since the pH increases non-uniformly during cooking. In as much as the advantageous effect of orthophosphate on juice retention has been attributed to an increase in pH *(3)*, it was considered desirable to include a sample of meat containing sodium orthophosphate without pH adjustment. The salt used was Na_2HPO_4.

In experiments in which the synergistic effect of ascorbic acid and phosphate were studied, the ascorbic acid was added to the meat at a concentration of 0.1%. Sodium tripolyphosphate was used as the phosphate salt. The two were blended thoroughly before adding to the raw meat.

Assay techniques. Oxidative rancidity was measured according to the 2-thiobarbituric acid (TBA) test as outlined by Turner *et al. (14)* with a slight modification in that optical densities were read on the Evelyn photoelectric colorimeter using a 540 filter. Juice losses were measured as cc. of liquid drained from the cans immediately after cooking, excluding the melted fat, and calculated as per cent of the total weight of the meat. A Beckman pH meter was used for pH determinations.

RESULTS AND DISCUSSION

Fat oxidation. The data in Table 1 demonstrate that over a range of temperature varying from 60° C. to 85° C., TBA values of pork increased rapidly shortly after cooking. Raw samples did not change appreciably. This same effect was noted in other experiments of longer duration. Only slight changes in TBA values occurred in raw meat after one to 2 weeks storage whereas cooked samples consistently increased to high values which were maintained throughout the storage period. Adequate taste panel data for statistical analysis were not obtained, but from limited observations it was noted that judges detected off-flavors at TBA values above 0.5.

The oxidative reaction took place rapidly at all temperatures investigated. Within 2 hours the samples had reached TBA values which were above 0.5. The effect of sterilization temperatures was not investigated. Information on the rate of oxidative rancidity at these temperatures is needed because of its importance to the canning industry.

It was of interest to determine whether or not the rapid increase in TBA values occurred in meats other than pork. Beef, chicken, lamb, and veal were prepared and assayed for oxidative rancidity using the technique which had been used for pork. Table 2 shows that the oxidative change which occurs in cooked meats stored at refrigerator temperatures (5–7° C.) apparently is not confined to pork. Here again, it is demonstrated that the reaction proceeds in cooked meats whereas raw meats do not show the change. The oxidative process is not limited to meats containing a relatively high percentage of unsaturated fatty acids (chicken and pork), but can occur in beef and lamb which contain fewer polyunsaturated fatty acids, and even in veal containing relatively small amounts of fat.

TABLE 1

Effect of cooking on TBA values of refrigerated pork

Internal cooking temperature	Storage intervals		
	0	2 hrs.	24 hrs.
Raw	.145	.109	.110
60° C.	.372	1.70	2.47
70° C.	.358	1.48	3.46
85° C.	.259	2.00	3.68

TABLE 2

Effect of cooking on rancidity in various meats

Sample	TBA values at various storage intervals		
	0	7 days	9 days
Beef:			
Raw	.516	.629	..... *
Cooked	.878	4.59	4.59
Chicken:			
Raw	.171	.213	..... *
Cooked	.921	4.59	4.59
Lamb:			
Raw	.406	.491	..... *
Cooked	.939	6.24	6.34
Veal:			
Raw	.475	.398	..... *
Cooked	.870	4.78	3.95

* Spoiled.

In earlier studies *(18, 21)* on pork these oxidative changes were not observed when peroxide determinations were used as a method of assay. No great increases in peroxide values were observed after freezing cooked meat. Although palatability ratings decreased, rancidity was not thought to be an important factor in organoleptic changes. It is possible that the TBA test measures oxidative changes which do not appear in peroxide determinations made on the extracted fat. The TBA test is a sensitive test for the decomposition products of highly unsaturated fatty acids *(7)* and can be performed directly on the meat tissue without the necessity of extracting the fat. It might, therefore, be expected to measure the oxidation of highly unsaturated protein bound phospholipids, not extractable by ordinary fat solvents. The activity of such compounds in producing off flavors in various foods has been recently reviewed *(8)*. Their role in meat is still obscure. Comparisons of TBA and peroxide values were not made on these meat samples. Therefore, it is impossible to state at the present time that these predicted differences actually exist.

The mechanism of the catalytic effect of heat on fat oxidation in meat tissue is not clear. Metal catalysis may be involved in the reaction. The accelerating effect of copper and iron on oxidation of fats has been well established. Heme pigments probably play an important role also. Watts *(20)* has reviewed evidence for a reaction between unsaturated fats and heme pigments which accelerates both rancidity and loss of color. The importance of heme catalysis in fat rancidity has been demonstrated further by Tappel *(13)*. Little is known at the present time about conditions under which heme catalysis becomes important in the preservation of meat. Possibly denaturation of proteins during heating may free phospholipids and thus make them more susceptible to oxidative attack.

Effect of phosphates. Figure 1 shows the effect of phosphates on TBA values in pork. These additives seem to offer a possible solution to the problem of

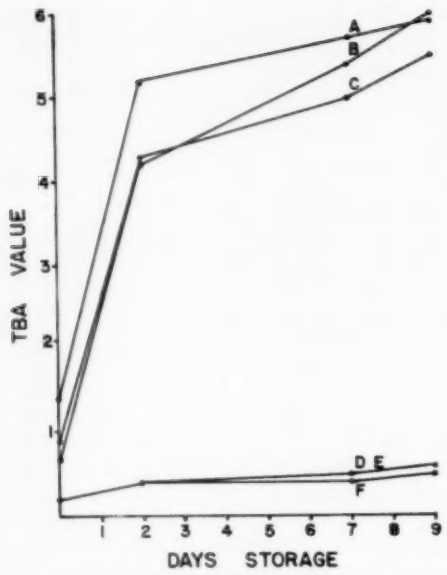

Figure 1. Effect of phosphates on TBA values of refrigerated cooked pork.
Legend: A. Control; B. Orthophosphate, no pH adjustment; C. Orthophosphate; D. Tripolyphosphate; E. Hexametaphosphate; F. Pyrophosphate.

oxidative rancidity. Orthophosphate was the only salt used which gave no protection with or without pH adjustment. After nine days storage it was no better than the control. Meats containing the other three phosphates, pyro-, tripoly-, and hexameta-, had TBA values within the range of acceptability after seven days storage.

Polyphosphates have been suggested as antioxidants for fats in heterogeneous systems *(9, 22)*. Since they are known to be metal sequestering agents, this may explain their antioxidant activity.

One of the outstanding effects of heat on meat tissues is loss of juice. Brissey's patent *(3)* suggests that orthophosphates can be used to decrease such losses. He attributed the effect to the alkaline pH. In order to test the hypothesis that the effect can not be attributed to pH alone, phosphate salts in aqueous solution were adjusted to pH 6.4 as described under Methods and Materials. The salts were added to the meat in the desired ratio and the pH taken before and after cooking. The percentages of juice losses and the corresponding pH's are given in Table 3. Although one may observe that the sample of meat which had the highest pH also had the least juice loss, this figure is only slightly below the 15.2% recorded for the sample containing sodium tripolyphosphate. Phosphates, in general, exerted an effect on juice retention in samples having similar pH values. Cooking losses were greater in the control than in samples containing ortho-, pyro-, or tripolyphosphates. These data on juice retention are not sufficiently extensive to serve as a basis for testing the hypothesis that pH and ionic strength are the main factors contributing to juice loss.

TABLE 3

Effect of phosphates on juice loss

Compound	pH		Per cent juice loss
	Raw	Cooked	
Control	5.87	6.10	19.2
Sodium orthophosphate, no pH adjustment	6.60	6.54	13.6
Sodium orthophosphate	6.02	6.10	16.0
Sodium pyrophosphate	5.97	6.00	16.8
Sodium tripolyphosphate	6.19	6.18	15.2
Sodium hexametaphosphate	5.82	5.97	17.6

As stated earlier, MIB has approved the use of phosphate additives in cured meat products only. However, the information presented here suggests other possible applications. Phosphate salts could be added easily to meat loaves and patties, stews, large cuts of meat cooked in liquids, or braised cuts to improve the quality of refrigerated or frozen cooked meats. As will be pointed out in a later paper, juice loss and the oxidative problem are greater in these meats than in cured products.

Synergism of phosphates and ascorbic acid. The effect of ascorbic acid is shown in Table 4. It accelerated rancidity when used alone, but a combination of ascorbic acid and tripolyphosphate was a powerful antioxidant. Ascorbic acid thus appears to act synergistically with phosphates to protect against rancidity. It also has been shown to accelerate rancidity in aqueous fat systems *(11)*. However, Lehmann and Watts *(9)* observed that when ethylenediaminetetraacetic acid or polyphosphates, both metal chelators, were added with ascorbic acid, it then became an antioxidant. Kelley and Watts *(6)* suggest that a copper-ascorbic acid complex may be the causative agent.

TABLE 4

Synergistic effect of ascorbic acid and tripolyphosphate on TBA values of cooked pork

Sample	TBA values at various storage[1] intervals		
	0	24 hrs.	18 days
Control	1.0	4.9	5.7
0.1% ascorbic acid added	.4	1.0	6.7
0.5% tripolyphosphate added	.3	.3	2.3
0.1% ascorbic acid and 0.5% tripolyphosphate added	.4	.4	.4

[1] Refrigerator storage (5–7° C.) was used.

SUMMARY

Oxidative rancidity in cooked meat products has been measured by the 2-thiobarbituric acid reagent. Rancidity occurred in all meats tested and was not limited to those containing many polyunsaturated fatty acids. TBA values surpassed the limit of acceptability within a few hours after cooking, but such increases were not observed in raw meat.

The antioxidant effect of several phosphates salts was studied. Pyro-, tripoly-, and hexametaphosphates had a protective effect whereas orthophosphate did not. Ascorbic acid acted synergistically with phosphates to protect against rancidity in meats. All phosphates decreased cooking losses.

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Heat Inactivation of Sweet Corn Peroxidase in the Temperature Range of 210° to 310° F.

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In an effort to improve the quality of canned vegetables, processors are adopting high-temperature, short-time methods of sterilization. It has generally been assumed that if the heat process given a low acid food at temperatures of 240° to 250° F. is adequate to destroy bacterial spores then the process will be more than adequate to inactivate enzymes associated with quality deterioration of the product.

Guyer and Holmquist *(4)* have shown that the enzyme peroxidase in peas has a high thermal resistance relative to other enzymes and some bacteria and that the activity of this enzyme may regenerate after heat inactivation. Farkas, Goldblith, and Proctor *(3)* reported a F_{250} value of 4 to 6 minutes and a z value of 48° F. for peroxidase in pea extract. They also found regeneration of enzyme activity after storage of the heated extract. Esselen and Anderson *(2)* reported on the heat inactivation of peroxidase in a number of vegetables. The F_{250} and z values for this enzyme showed considerable variation among vegetables and between varieties of a given vegetable. The highest values found were 14.5 minutes for F_{250} and 75.3° F. for z. In the case of peroxidase in one green bean sample the z value and F_{250} value were 69.1° F. and 0.51 minutes, respectively, for inactivation when samples were analyzed immediately after heating. The data for heat treatment required to prevent regeneration in the green bean sample showed a z value of 44.4° F. and a F_{250} value of 3.7 minutes. The z value commonly accepted for the most resistant bacterial spores is about 18° F. It is not uncommon for a vegetable peroxidase to have a z value above 40° F. Therefore, in heating above a certain critical temperature longer process times will be necessary to irreversibly inactivate such an enzyme than to destroy bacterial spores. Under such conditions, enzyme inactivation would be a more suitable indicator of an adequate thermal process than would be bacterial spore destruction.

That peroxidase activity is in some way associated with the development of off-flavors in processed foods has been indicated by several investigators. Guyer and Holmquist *(4)* noted that the development of off-flavor in canned high-temperature, short-time sterilized peas followed the regeneration of peroxidase activity. The degree of off-flavor increased as the amount of enzyme activity increased. Off-flavors in fresh pack pickles have also been closely associated with peroxidase activity *(6, 7, 8)*. Horseradish peroxidase, when added to fresh pack pickles in which all the naturally occurring enzyme had been destroyed, resulted in the development of off-flavors similar to those found in samples in which all the naturally occurring enzyme had not been inactivated.

Before enzyme activity can be used to determine process times, the heat inactivation properties of the enzyme must be experimentally determined. Because of the variation of these properties of peroxidase in different products (2), it is necessary to determine them for each. Corn is currently being processed using high-temperature, short-time procedures. Therefore, this study was undertaken to determine experimentally the heat inactivation properties of sweet corn peroxidase in a partially purified preparation and in raw whole kernel corn.

EXPERIMENTAL METHODS

Preparation of samples. Whole kernel corn was prepared by mechanically removing the kernels from freshly harvested sweet corn. The kernels were washed in a rod reel washer and used immediately.

The preparation of partially purified sweet corn peroxidase was essentially that used by Reddi, Esselen, and Fellers in isolating a partially purified apple peroxidase *(9)*. Because a large proportion of the corn starch was carried through the preparation of the enzyme, an additional step was used to remove most of this contaminant. The enzyme was precipitated during overnight storage at 40° F. from a 2.5 M $(NH_4)_2$ SO_4 solution of the partially purified preparation. It was separated by centrifuging at 3000 r.p.m. for 10 minutes and dried under 28 to 29 inches Hg. vacuum at 70° F. for 24 to 30 hours. This partially purified precipitate of sweet corn peroxidase was approximately 30 times as active as raw whole kernel corn and was stable at 0° F.

Heating of samples. Whole kernel corn was heated in thermal death time (TDT) cans (208 x 006) of the type described by Townsend, Esty, and Baselt *(12)*. Approximately 13 g. of raw cut sweet corn was added to each can. The interstices between kernels were filled with distilled water. The cans were sealed under vacuum by a Pacific Number 1 closing machine using a specially constructed mount for holding the small cans so that cans equipped with a thermocouple could be sealed.

The TDT cans were heated in a retort constructed in this laboratory. It was equipped with vacuum, steam, and cooling water lines, and a stuffing box for entrance of thermocouple wires. A maximum number of 14 cans held in a wire basket were heated at one time in the retort. A vacuum of about 25 inches Hg. was drawn before steam was admitted to facilitate rapid temperature rise in the retort and uniform heating of the cans. The cans were cooled rapidly by flooding the chamber with water.

Samples of partially purified enzyme were heated in Pyrex capillary tubing. The tubing had a 3 mm. O. D. and an approximate wall thickness of 0.6 mm. It was cut into lengths of 6 inches and one end was sealed. Five grams of the enzyme preparation was made to a volume of 100 ml. with distilled water and 0.25 ml. of this solution was added to each tube by means of a 1 ml. tuberculin syringe equipped with a 6½ inch hypodermic needle. The solution was added so that an air space of from ½ to 1 inch was left in each end of the tube. The open end of the tube was then sealed instantly with an oxy-acetylene flame.

Tubes were heated in an agitated oil bath with temperatures

controlled to ±0.1° F. A special carrier was constructed from stainless steel to hold 9 tubes while they were being heated. The heated tubes were cooled by plunging the tube carrier in a bath of cool tap water.

Heat penetration studies. Determinations were made of the penetration of heat into the TDT cans and into the capillary tubes. For the TDT cans special bare junction type Ecklund thermocouples were used. One of the kernels inside the TDT can was impaled on the junction of this thermocouple so that temperatures inside the kernel were determined. Readings were recorded on a Minneapolis-Honeywell 12-point recording potentiometer. This thermocouple was connected to all 12 points of the instrument so that each point recorded its temperature. Under these conditions the instrument produced a smooth curve from which the time required for the kernel interior to reach retort temperature could be determined.

Heat penetration into the capillary tubes was studied using a fiberglass insulated thermocouple lead wire. The wire was sealed into a capillary tube as shown in Figure 1. Several inches of insulation were removed from the wire. The bare constantan wire was enclosed in a length of very small diameter glass capillary. The ends of the copper and constantan wires were fused and the wires inserted into a H.I.T. capillary of the type used for this study. The end of the tube through which the wires passed was then sealed by heat. The bare wires extending from the tube were covered with vinylite cement and wrapped with electrical tape. Through the open end of the capillary enough enzyme solution to cover the fused junction of the thermocouple wire was added and the end sealed. Temperature readings were made with a Leeds and Northrup portable potentiometer.

Corrections for heat penetration lag time were made by the graphical method of Bigelow *et al. (1)*.

Storage of samples. TDT cans were stored at 36°, 75°, and 100° F. for 0, 2, and 7 days. Capillary tubes were stored at 75° F. for 0 and 72 hours.

Analysis for enzyme activity. Peroxidase activity of the whole kernel corn heated in the TDT cans was determined spectrophotometrically using the method of Vetter, Steinberg, and Nelson *(13)*.

A few modifications were made in this procedure in order to determine the enzyme activity of samples heated in the capillary tubes. The tube was broken on each end and the enzyme solution allowed to run out into 7.75 ml. of buffer. The tube was broken into small pieces and added to the solution with 0.5 ml. of 1% orthophenylenediamine, 0.5 ml. of 0.3% H_2O_2, and 1 ml. of a saturated solution of $NaHSO_3$. After color development the solution was centrifuged at full speed for three minutes in a clinical centrifuge. The supernatant was decanted into a colorimeter tube and analyzed as described *(13)*.

RESULTS AND DISCUSSION

Correction factors for heat penetration lag. Studies on the penetration of heat into the capillary tubes at temperatures from 210° to 300° F. showed that 16 seconds were required in each case for the interior of the tube to reach bath temperature. Corrections for heating time for this lag of heat penetration were determined using z values obtained from a graph of our uncorrected heat inactivation data for this enzyme. These z values were 52° F. and 186° F. for temperatures below and above 270° F., respectively.

At temperatures of 210°, 220°, and 230° F. it was found that the 16 second come-up time had negligible equivalent destruction value for the temperature employed. Therefore, the full 16 seconds were subtracted from heating times at these temperatures. At temperatures of 240° F. and above the lag time of 16 seconds had an equivalent destruction value of 2 seconds for each temperature studied. Therefore, 14 seconds were subtracted from all heating times at these higher temperatures.

Studies on the penetration of heat into a kernel of sweet corn in a TDT can heated in the TDT retort showed that 110, 145, and 215 seconds were required to reach processing temperatures of 248°, 275°, and 300° F., respectively. Equivalent destruction values for these lag times were determined using corrected z values obtained from data on heat inactivation of this enzyme in capillary tubes. Results of this study are summarized in Table 1. All subsequent process times were corrected for this lag in heat penetration by subtracting the appropriate correction term.

Heat inactivation of partially purified sweet corn peroxidase. Results of studies on the heat inactivation of partially purified sweet corn peroxidase are shown graphically in Figures 2 and 3. Figure 2, the heat inactivation curve obtained when samples were analyzed for enzyme activity immediately after heating, shows two straight lines intersecting at about 270° F., a F_{250} value of 1.33 minutes and z value of 42° F. for temperatures below 270° F. and a F_{300} value of 0.22 minutes and z value of 100° F. for temperatures above 270° F. The heat inactivation curve based on analysis after 72 hours storage at 75° F., Figure 1, has an inflection point at about 260° F., a F_{250} of 1.78 minutes and z value of 40° F. for temperatures below 260° F., and a F_{300} value of 0.26 minutes and z value of 86° F. for temperatures above 260° F. The 300° F. reference was arbitrarily chosen as a convenient temperature which could be used to construct that portion of the heat inactivation curve at temperatures above the inflection point.

The change in slope of the heat inactivation curve for peroxidase as temperatures exceed about 265° F. is of particular interest to processors of sweet corn using high-temperature, short-time sterilization techniques. The enzyme becomes more resistant to heat at temperatures above this inflection point. A process calculation based on the low temperature portion of the curve would indicate that 6 seconds of effective heating time at 300° F. would suffice (Figure 3). However, the curve for temperatures above 270° F. shows that an effective heating time of about 20 seconds is needed to destroy the enzyme at 300° F. Therefore, processes at these high temperatures should be based on data obtained at high temperatures.

Heat inactivation of enzyme in corn. The inactivation of peroxidase in whole kernel sweet corn which has been sealed in TDT cans and heated in the TDT retort at 3 temperatures was studied. Samples were analyzed for enzyme activity after 2 and 7 days' storage at 36°, 75°, and 100° F. as well as soon after processing. Since process times were varied by increments of 50 or 100 seconds, the data are approximate.

TABLE 1

Correction terms applied to samples heated in the TDT retort

Retort temperature	Time to reach retort temperature	Equivalent destruction value for come-up time	Correction term
(° F.)	(seconds)	(seconds)	(seconds)
248	110	50.6	59.4
275	145	99.8	45.2
300	215	146	69.0

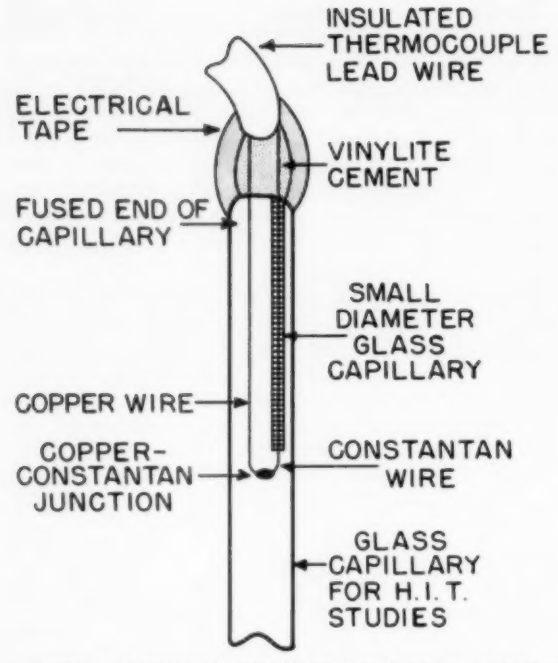

Figure 1. Diagram of assembly used to determine heat penetration into capillary tube containing enzyme preparation.

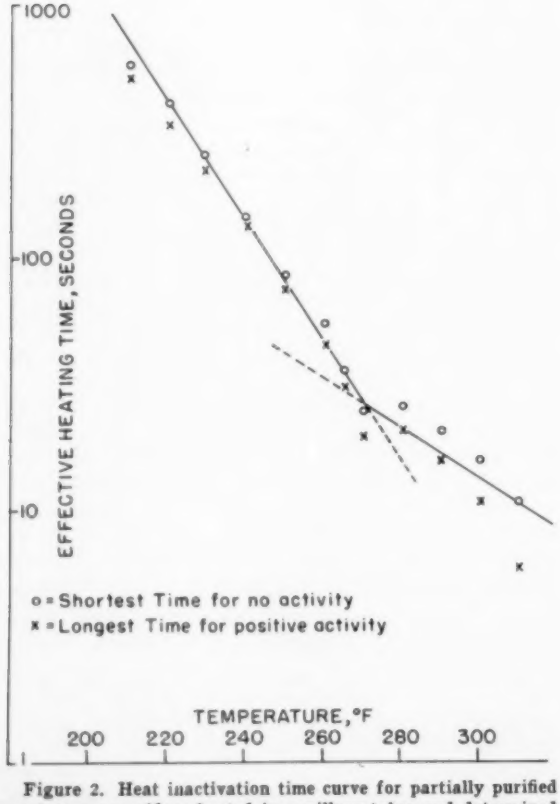

Figure 2. Heat inactivation time curve for partially purified sweet corn peroxidase heated in capillary tubes and determined immediately after heating.

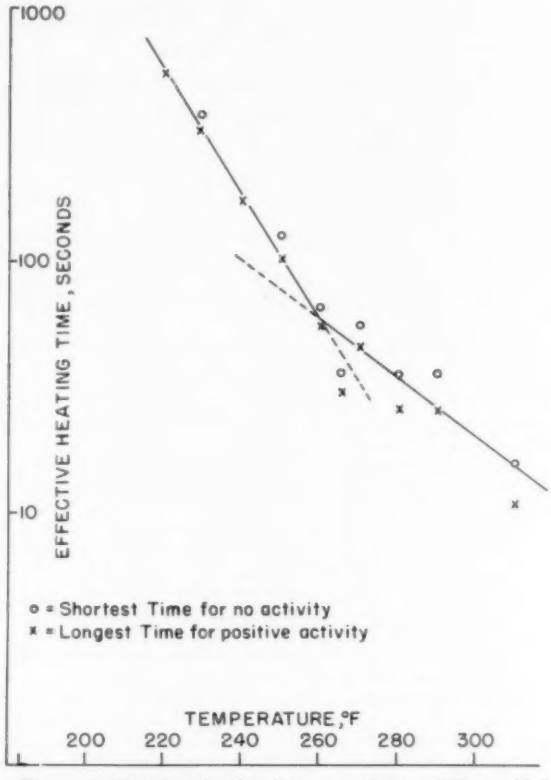

Figure 3. Heat inactivation time curve for partially purified sweet corn peroxidase heated in capillary tubes and determined after 72 hours storage.

However, several interesting observations were made.

The enzyme regenerated after inactivation by heating. The amount of this enzyme regeneration was found to increase with increasing storage temperature. Data obtained after 7 days' storage at the 3 temperatures were essentially the same as those obtained after 2 days' storage. Therefore, it appears that regeneration of enzyme activity was completed within 2 days. Although the results with partially purified enzyme indicated a similar regeneration (Figures 2 and 3), the differences were not as marked.

Times required for heat inactivation under the several conditions for this experiment are plotted on a logarithmic scale in Figure 4. This illustrates the general observations. Thus, at a processing temperature of 300° F. the equivalent heat inactivation time was only 30 seconds if the sample had not been stored, 90 seconds if stored for 2 days at 36° F. and 130 seconds if stored the same time at 100° F.

It is of interest to compare the heat inactivation of peroxidase in a partially purified state (Figures 2 and 3) with its destruction in whole kernel corn (Figure 4). The data indicated a greater heat resistance for the enzyme when in whole kernel corn. Thus, at a processing temperature of 275° F. and storage of samples at 75° F. before analysis, the heat inactivation times were 150 seconds in case of the corn and 40 seconds for the enzyme preparation.

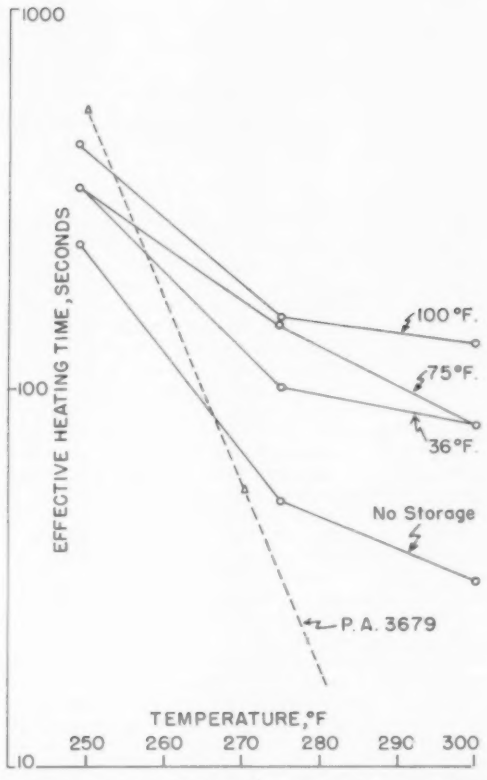

Figure 4. Heat inactivation time curves for peroxidase in raw whole kernel sweet corn heated in thermal death time cans and stored for 2 days at three temperatures as compared to curves for unstored control and a bacterial spore.

Of great economic importance is a comparison of heat inactivation properties of sweet corn peroxidase enzyme and a thermophilic microorganism. To facilitate this comparison a thermal death time curve for N.C.A. organism P.A. 3679 in corn at pH 6.8 is included in Figure 4. A z value of 21° F. and a F_{250} of 9.0 minutes *(11)* were used to draw this line. It can be seen that at temperatures of 250° F. and slightly above, the microorganism is more resistant to heat than the enzyme. However, at temperatures above about 260° F. the enzyme is more resistant. Therefore, high temperature processes based on the destruction of a heat resistant microorganism may leave residual enzyme activity in the canned product. Since peroxidase has been associated with off-flavor development in canned vegetables, this may lead to quality deterioration. Thus, it would seem advisable to determine high temperature sterilization processes on the basis of enzyme inactivation as well as the destruction of microorganisms.

SUMMARY

Studies were made on the heat inactivation of sweet corn peroxidase in capillary tubes using a partially purified preparation and in TDT cans using whole kernel corn. Temperatures in the range of 210° to 310° F. were used. Data on the penetration of heat into the capillary tubes and the TDT cans are given and were used to correct process times.

Heat inactivation of sweet corn peroxidase was found to be characterized by relatively high F_{250}, F_{300}, and z values and a tendency to regenerate activity after storage. F_{250} and F_{300} values were higher when the enzyme was heated in whole kernel corn than when heated in a partially purified solution. In the case of the latter, F_{250} values found were 1.33 and 1.78 minutes before and after 72 hours storage, respectively. F_{300} values were 0.22 and 0.26 minutes before and after storage. When heated in whole kernel corn, F_{250} values for the enzyme were approximately 3.7 to 7.0 minutes and F_{300} values ranged from 0.5 to 2.1 minutes depending on time and temperature of storage.

Heat inactivation curves for the enzyme indicated an inflection point at about 270° F. The curves showed higher z values above this temperature. The z values found ranged from 40° F. to well over 100° F. depending on environment during heating, temperatures of inactivation, and time and temperature of storage after heating.

Acknowledgment

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Effectiveness of Commercial Papain in Meat Tenderization [a,b]

(Manuscript received May 13, 1957)

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PAPAIN, A PROTEOLYTIC ENZYME present in the milk juice of the tropical plant *Carica papaya*, is the active ingredient in most meat tenderizers. Tenderization is effected by the proteolytic activity of this enzyme which breaks apart the muscle fiber proteins and, to some extent, by hydrolysis, the connective tissue of meat. Enzyme-treated meats could be prepared by short methods and, as a result, the price difference between tender and less tender cuts of meat should be lessened.

Hay, Harrison and Vail *(6)* studied the effects of papain on certain muscles of beef subjected to various conditions of pretreatment and cooking. They observed very little effect on palatability as a consequence of papain action but a significant tenderization in the majority of meat cuts studied. These findings have become widely known. Due to the public demand for meat tenderizers, an increasing number of commercial preparations containing this enzyme have appeared on the market. Despite the increase of available meat tenderizers which incorporate papain as the active component, there has been very little investigation of the effectiveness of these preparations. There is, of course, considerable information available pertaining to the enzyme papain itself *(8, 14)*, but these data are mainly concerned with assay procedure and the factors affecting enzyme activity.

Many studies have shown that the physical and chemical properties of the enzyme can be varied by methods of extraction *(10, 12, 13)*. It has been shown further that the rate and type of hydrolysis depends upon the incorporation and type of activator *(1, 16)*, pH *(10)* and temperature *(3, 4, 5, 9)*.

Since the nature and the action of the enzyme can be varied, a study of the relative efficiency of a number of locally available commercial papain meat tenderizers was undertaken under different conditions.

METHODS AND MATERIALS

Enzyme solutions. (a) *Papain.* Three papain preparations of unknown purity in addition to a papain preparation from the National Formulary were used in this study. The papain preparation from the National Formulary, designated number 17, was used as the reference standard throughout the experiments. Five per cent solutions of the enzyme, by weight, were found to be satisfactory for stock solutions. The papain was weighed to the nearest tenth of a milligram and made to volume with boiled distilled water.

For the milk clotting assay, dilutions were made up from stock solutions to yield concentrations of 2.5, 5.0, 7.5 and 10.0 mg. papain per ml. These solutions when activated, would clot the milk solution in 30 seconds to 3 minutes. Additional determinations on the activated reference papain were made at concentrations of 0.5, 1.0, 1.5, and 2.0 mg. per ml. In experiments with milk substrates, triplicate samples of the enzyme solutions were used in both activated and unactivated form. Activation was accomplished by bubbling hydrogen sulfide gas through the papain suspensions for six minutes.

For the purpose of the beef digestion assay, a dilution of each of the papains was selected which would contain the equivalent of 1.5 mg. of the activated reference papain preparation per milliliter or which would clot the milk in approximately 2.5 minutes. Activated papain solutions were not used with beef since this substrate contains natural activators. One-milliliter portions of these solutions were used throughout the experiments. Two and 3 ml. portions were used in the study of the relation between amount of enzyme and amount of digestion.

(b) *Tenderizers.* Since there was no way of knowing the amount of papain present in the commercial tenderizers, preliminary work was done to determine the amount of tenderizer to be used. With most powdered tenderizers, concentrations of 100, 200, 300, and 400 mg. per ml. would, when activated, clot milk in 0.5 to 3.5 minutes. With activated liquid tenderizers, if measurable activity was observed, 60, 80, and 100% solutions by volume as purchased clotted milk within the same time range. These concentrations were used for computation of equations and units of milk clotting activity per gram of tenderizer. As with the papain solutions, a concentration of tenderizer 1 ml. of which, when activated, would clot 10 ml. of milk in 2.5 minutes was selected for the beef digestion assay.

Assay methods. (a) *Milk Clotting.* The method of Balls and Hoover *(2)* as modified by Hinkel and Alford *(7)* was used in the study of the milk clotting ability of papain alone and commercial tenderizers in which papain was the active hydrolytic agent. Buffered milk solutions were made up as required and stored at 3° C. under toluene. Ten milliliters of milk were pipetted into 15 x 127 mm. test tubes. The tubes were fitted with rubber stoppers and immersed in a 40° C. constant temperature bath. When the milk solution in the tubes had reached the bath temperature, 1 ml. of the papain or tenderizer was rapidly added by being blown from a 1 ml. wide tipped pipette. Simultaneously, an electric stopclock was started. The tubes were then shaken in the bath sufficiently to give a film of the test suspension on the upper sides of the tube and the film was allowed to drain. Just prior to clotting, the milk layer thickened and failed to drain from the walls of the tube. The actual clotting was considered to take place at the instant the film of milk became discontinuous and pebbly. The length of time in minutes from the addition of the papain solution to the clotting of the milk was recorded. The equations of the lines representing the relations between the reciprocal of clotting time and concentration of enzyme were computed by the method of least squares from values obtained for both unactivated and activated papain and commercial tenderizers incorporating papain. These equations were used to determine the unit of milk clotting activity, defined as the amount of papain or tenderizer necessary to clot 10 ml. of milk at 40° C. in one minute. From these values, the units of milk clotting activity per gram of enzyme preparation were calculated.

(b) *Beef Digestion.* Lack of reproducibility of meat substrate was believed to be one of the major problems in the beef digestion method. The following experiments were run in an attempt to achieve reproducibility and simplicity. Experiments were run with all substrates at 40° C. and with substrate 3, at 20° C., 40° C., 60° C., and 80° C.

1. Slurry Substrate. Frozen ground beef from a single lot was thawed and blended three minutes in a Waring blender

[a] Presented before the Seventeenth Annual Meeting of the Institute of Food Technologists, Pittsburgh, Pennsylvania. May 12, 1957.

[b] Journal Series Paper No. 1750, approved by the Director of the Missouri Agricultural Experiment Station.

adjusted to slow speed. One quarter pound of beef to 250 ml. of distilled water was used to make up the mixtures. Ten milliliter portions of the beef slurry were pipetted from the blender to 15 x 127 mm. test tubes. The tubes were placed in a water bath. When the temperature of the slurry reached the temperature of the water bath, the papain or commercial tenderizer solution was added, the mixture shaken and incubated 2 hours. The mixture was then transferred to a funnel equipped with rubber tube and pinch clamp, allowed to stand 5 minutes, and the pH determined with a Beckman pH meter with external electrodes. The range of pH obtained after digestion with the different enzymes was from 5.95 to 6.58. Five milliliters of 38–40% formaldehyde solution freshly neutralized to phenolphthalein was added and the mixture was allowed to stand 15 minutes. The solution was then adjusted to pH 8.2–8.3 with 0.1N NaOH. Volume corrections were made for enzyme and water blanks which were run in duplicate for each day's experiment. The amount of digestion was recorded in terms of milliequivalents of NaOH (and hence of carboxyl groups freed in digestion) per gram of papain or tenderizer.

Comparable slurries but containing no enzyme were run as control blanks, and titrated in a similar manner.

2. Centrifuged Liquid Substrate. The slurry substrate method was modified by increasing the blending time to 4 minutes and centrifuging for 6 minutes. The supernatant liquid, freed from fat and other solids by straining, was used as the digestion substrate. The range of pH obtained after digestion of this substrate was from 4.93–6.82.

3. Meat and Water Substrate. A substrate was prepared by weighing out 3.1 g. of lean ground meat to 6.9 ml. of water into test tubes for each individual digestion. This method eliminated the need for blending, centrifuging and straining.

After the digestion period the centrifuged liquid substrate and the meat and water substrate were transferred to small beakers instead of a funnel. Use of the beaker permitted more thorough stirring. The range of pH obtained after digestion of this substrate was from 5.19–6.02. The amount of digestion recorded in terms of milliequivalents of sodium hydroxide was once again used as a criterion of enzymatic activity.

One, 2, and 3 ml. amounts of active enzyme solutions were used with the centrifuged substrate and the meat and water substrate in a study of the type of relationship between quantity of enzyme and amount of digestion.

RESULTS AND DISCUSSION

A. Proteolytic Activity of Pure Papain and Tenderizers Containing Papain Using a Milk Clotting Method

Experimental results are in agreement with data previously reported which indicate that a linear relationship exists between the reciprocal of time and concentration of activated and unactivated enzyme solutions. Results of plotting reciprocals of time required for clotting milk versus varying concentrations of 4 pure papain preparations are shown in Figure 1.

The equations of the lines representing the relations between the reciprocal of clotting time and concentration of enzyme were computed as described under assay methods. These equations were used to determine the units of milk clotting activity which are shown in Table 2. It was found that using low concentrations of the reference papain, 17, the equation for the line would have bisected the axis of enzyme concentration at a finite value. Balls and Hoover *(2)* obtained similar results and concluded that the milk contained a substance capable of inhibiting the enzyme.

The 4 pure papain preparations ranged in milk clotting activity from 14 to 107 units before activation and from 117 to 203 units after activation. (Table 2). The reference papain, 17, had the lowest milk clotting

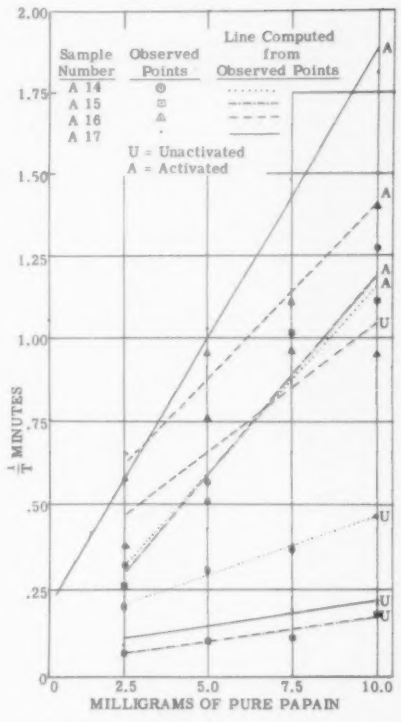

Figure 1. Relation between clotting time and amount of enzyme for four pure papain samples.

action before activation, but the highest after activation.

Units of activity per gram of the meat tenderizers as computed from the milk clotting time may be compared with the activity of pure papain 17 by using columns 3 and 5 of Table 2. Powdered tenderizers varied in their activity from negligible amounts to approximately three per cent in relation to activated pure papain 17. There was the possibility that salt and spices interfered with the activity in test sam-

TABLE 1

Description of commercial tenderizers

Sample number	pH	Ingredients in addition to enzyme extract listed on label
Liquid		
1	4.48	Benzoate of soda.
2	7.53	Monosodium glutamate, vegetable protein, vegetable juices, cane sugar, invert sugar, sodium benzoate, sodium bicarbonate and spice and flavorings.
3	3.85	Fruit acids and sodium benzoate.
4	4.11	Salt and sherry wine.
5	3.85	Fruit acids, sodium benzoate and coloring.
6	5.33	Salt and glycerine.
7	7.85	Salt and glycerine.
8	4.75	Smoked salt, hydrated plant proteins, monosodium glutamate and sodium benzoate.
9	5.99	Salt, vegetable juice and monosodium glutamate.
Powdered		
10		Salt, spices and dextrose.
11		
12		Salt, dextrose, hydrated plant protein, processed wheat, tri-calcium phosphate, mushroom powder and flavoring.
13		Salt, dextrose, monosodium glutamate, processed wheat, tri-calcium phosphate and flavorings.

TABLE 2

Enzymatic activity of pure papains and papain tenderizers at 40° C.[1]

Sample description	Milk clotting		Beef digestion milliequivalents per gram	Comparison of milk clotting and beef digestion (meat slurry) per cent active papain in relation to pure papain 17	
	Before activation units per gram	After activation units per gram		Milk clotting	Beef digestion
Pure papain powder					
14	40	117	55	58	70
15	14	119	50	59	64
16	107	166	52	82	67
17	16	203	78	100	100
Powdered tenderizer					
0	2.15	4.52	1.40	2.2	1.8
10	a	0.31	0.55	0.2	0.7
11	0.09	0.85	0.58	0.4	0.7
12	0.53	5.52	2.10	2.7	2.7
13	0.42	5.77	2.40	2.8	3.1
Liquid tenderizer					
1	0.14	1.49	0.48	0.7	0.6
2	a	a	0.00	0.0	0.0
3	0.08	1.67	0.42	0.8	0.5
4	0.08	2.30	0.99	1.1	1.3
5	0.07	1.66	0.36	0.8	0.5
6	0.02	0.42	0.33	0.2	0.4
7	a	a	0.00	0.0	0.0
8	0.04	0.24	0.17	0.1	0.2
9	a	a	0.05	0.0	0.1

a = negligible.
[1] First series of experiments.

ples 10 and 11. However, samples 12 and 13 were observed to have a large proportion of salt and spices and relatively high activity.

Liquid preparations exhibited lower activity than powders. The activity of the liquid tenderizers in relation to papain 17 varied from an amount too small to measure by this method to 1.1% of activated papain 17. Pulley and Von Loesecke *(11)* analyzed 2 liquid meat tenderizers prepareed in Florida and reported that Sample A had 0.11 activity units and Sample B had 0.40 activity units on activation. Results obtained in the present experiment showed close agreement with these data.

The pH of the liquid tenderizers varied from 3.85 to 7.85. The 3 tenderizers with pH of 5.99 and above showed no measurable milk clotting activity, even after activation whereas the two with pH of 4.75 and 5.33 showed only slight activity. Those tenderizers with pH of 4.48 and below showed the highest activity.

Those tenderizers which had instructions for the cooking of meat or vegetables shortly after application of the tenderizer, appeared to have the highest milk clotting activity; those which were to be in contact with the meat or vegetables from 10 to 12 hours had a much lower activity.

B. Proteolytic Activity of Pure Papain and Tenderizers Containing Papain Using a Beef Digestion Method

In general, when the temperature was 40° C., enzymatic activity measured by beef digestion was comparable to that measured by milk clotting method (Table 2) but more variation was obtained among replicates no matter what modification of substrate was used.

Table 2, columns 4 and 6, shows beef digestion activity of both papain and tenderizer solutions when 1 ml. portions were used with beef slurry substrate. Activity in terms of milliequivalents per g. at 40° C. ranged from 50 to 78 for the pure papains. The other 3 papain samples had beef digestion activities from 64 to 70% of the reference papain which was again highest. The powdered tenderizers had activities of from 0.55 to 2.40 milliequivalents per g. or from 0.7 to 3.1% of the reference papain. The liquid tenderizers again showed the lower activities, from 0.0 to 0.99 milliequivalent per g. or from 0 to 1.3% of the reference papain.

Table 3 shows beef digestion activities of the pure papains using 3 meat substrates at 40° C. Papain 17 had the highest rate of beef digestion except in the case of the centrifuged substrate. Beef digestion activity was much lower and more variable for all papains with this substrate than with the other two. The meat-and-water substrate gave the highest activity values of the three. The results with this substrate apparently were no more reproducible than with the slurry but were more econvenient to use. Variation in relative activity of enzymes with the 3 substrates used may be due chiefly to differences in composition or particle size of the substrate. Additional work will be necessary in developing a uniform meat substrate. It is possible that a dried pulverized fat-free beef might be more homogeneous than the substrates used to date.

Effects of varying the concentrations of pure papains in beef digestion experiments are shown in Table 4. Again, probably due to lack of uniformity of substrate, ranges in values obtained for a given experiment are great. However, all 3 papains as well as the tenderizers tested showed increased beef digestion with increased concentration of enzyme, but less digestion per gram of enzyme.

When beef digestion activity was plotted against the logarithm of the enzyme concentration for both activated and non-activated pure papains studied, only a few of the lines representing the relationship were straight and they varied considerably from one determination to another, even with the same papain sample. This again was considered to be due at least in part to the variability of the beef substrate. When the average beef digestion activity of each pure papain was plotted against the enzyme concentration itself, the lines drawn for two of the three papains seemed to be quite straight. These same activities plotted against the logarithm of the enzyme concentration showed less linearity. Thus it was not possible in this study to confirm the theory of Hinkel and Alford *(7)*

TABLE 3

Comparison of beef digestion activity of pure papains at 40° C. using three modifications of substrate[1]

Papain sample	Beef digestion activity—milliequivalents per gram		
	Original meat slurry substrate	Centrifuged substrate	Meat-and-water substrate
14	56.5		
15	51.1	2.7	79.3
16	53.5	17.8	90.7
17	79.4	13.7	91.3

[1] One ml. enzyme solution.

TABLE 4

Comparison of beef digestion activity of pure papains at 40° C. using one, two, and three milliliters enyme solutions wtih meat-and-water substrate

Sample No.	One milliliter milliequivalents per gram	Two milliliters milliequivalents per gram	Three milliliters milliequivalents per gram
15	79.3	53.3	46.7
16	90.7	64.1	50.4
17	91.3	45.0	40.4

that beef digestion activity of papain is a function of the logarithm of the enzyme concentration. It would appear to be quite unusual from a theoretical standpoint if the activity of the enzyme were related linearly to the logarithm of the concentration of the enzyme. This type of relationship would usually hold only through a very limited range of enzyme and substrate concentration.

Tappel *(15)* has stated that the optimum temperature for papain digestion is between 60° and 80° C. The data plotted in Figure 2 are in agrement with his findings. As temperature increased, the activity of the enzyme preparation increased over the range studied. At 80° C. there is demonstrable activity even with those preparations which were completely inactive at 40° C. The optimal temperature for any enzymatic reaction is related closely to the time which that reaction covers. In general, the shorter the digestion time the higher is the optimal temperature for that reaction. Since a relatively short digestion periood was used in these experiments, it would be expected that the optimal temperature would be comparatively high. It is entirely possible that maximum tenderization takes place during the cooking process.

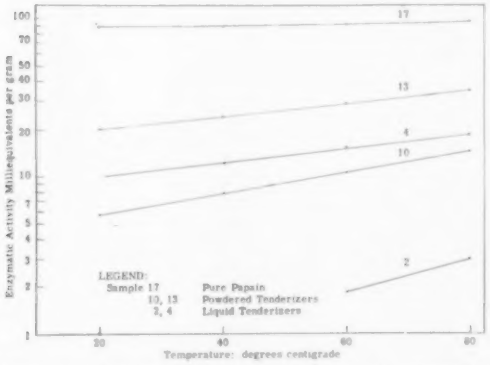

Figure 2. The beef digestion activity of representative papain preparations at 20°, 40°, 60°, and 80° centigrade.

SUMMARY AND CONCLUSIONS

The proteolytic activity of four pure papains and fourteen tenderizers containing papain was studied using both milk clotting and beef digestion methods. The linear relationship which had been reported between the reciprocal of milk clotting time and enzyme concentration was shown to exist under the conditions of this experiment for both pure papains and meat tenderizers. Likewise, as the temperature increased the activity increased but not enough work was done to define the nature of the relationship.

The milk clotting test was used as a standard method of assay to evaluate the beef digestion method. With the concentrations used in this experiment, the milk clotting and beef digestion methods were found to give similar estimates of proteolytic activity. The activity of the tenderizers ranged from none to approximately 3% of the activity of the reference papain when tested at 40° C. The relative activity of the tenderizers was increased at higher temperatures, however. The powdered tenderizers were found to have a higher activity than mostt liquid tenderizers by both the milk clotting and beef digestion methods. Two modifications of the beef slurry substrate were tested but did not give more results than the slurry, although one modification was more convenient to use. Although additional work needs to be done on development of a reproducible meat substrate, it seems possible that the beef digestion method could be adapted for the study of enzymatic activity and beef tenderization. However, it should be pointed out that there is not necessarily any relationship between the milk clotting test, the beef digestion method, and tenderness improvement as measured organoleptically. It is possible that tenderization may occur before the proteolytic effect is measurable. If so, organoleptic testing should be used for determination of tenderization.

TABLE 5

The infiuence of temperature on beef digestion activity of papain preparations[1]

Sample No.	Enzymatic activity at specified temperatures—milliequivalents per gram			
	20° C.	40° C.	60° C.	80° C.
Pure papain powder				
15	76.55	79.30	83.75	87.65
16	88.50	90.70	94.20	96.35
17	89.00	91.33	95.75	98.50
Powdered tenderizer				
10	5.75	8.50	10.75	14.35
11	13.25	17.90	21.15	26.84
12	19.00	23.10	29.33	35.68
13	20.25	24.20	29.63	35.32
Liquid tenderizer				
1	7.65	9.35	12.75	14.50
2	0.00	0.00	1.88	3.00
3	3.65	5.75	9.00	13.36
4	10.00	12.25	15.53	18.57
5	3.33	4.25	6.76	8.34
6	18.65	22.75	28.35	35.75
7	0.00	0.00	2.25	4.75
8	1.95	2.55	4.75	8.00
9	1.10	1.85	2.68	4.25

[1] Second series of experiments.

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Continuous Jelly Manufacture

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The continuous jelly process reported here is a result of work in recent years on utilization of fruits in the form of concentrated juices and purees. Since jelly manufacture always requires removal of water from single-strength juice, the use of concentrated juice as a starting material adds the advantage of lower shipping and storage costs to that of simplified jelly making by continuous processing.

Jelly making has historically involved preparation of a fruit juice, followed by cooking the juice in a kettle with sugar and, frequently, pectin, acid, and other minor ingredients until sufficient water has been removed to give a jelly with the proper consistency and sweetness. However, coking single-strength juice and sugar in an open kettle long enough to remove the amount of water required to produce a legal jelly damages the flavor to some degree. Not only are some volatile flavoring components removed with the water evaporated, but some scorched flavors are produced, under the high-temperature, long-time conditions employed. The color of the jelly is also damaged by boiling under these severe conditions, usually with formation of some brown color. Furthermore, quality control is difficult. Batches vary owing to difficulty of control of the end point of cooking, and because of variations in time of handling.

By using concentrated fruit juice for making jelly it is possible to obtain better color and flavor than with conventional cooking of single-strength juice. In modern concentrating equipment *(2, 4, 6)* color and flavor are preserved because product temperature and processing time are minimized. The short reheating required for making jelly causes little, if any, damage. Flavor is also preserved by use of volatile flavor recovery units prior to or during concentration of the juice *(8, 9)*. The concentrated volatile flavor solution which is recovered can be added back to the juice concentrate to strengthen the natural flavor. With some juices, however, especially grape, it may not be necessary to return volatile flavors because of their relatively small amount.

The difficulty of control of the jelly cooking process in a batch operation has given rise to several attempts at putting the process on a continuous basis. For example, in a process described by Baker and Phetepplace *(1)*, the juice, containing its natural pectin, was evaporated continuously in a vacuum pan. The concentrate was blended in heated tanks with sirup and more pectin, if needed, to make the jelly. An advantage of this procedure over ordinary methods is the low-temperature concentration of the juice in the vacuum pan, a modification which is becoming widely used at present. Some disadvantages are: single-strength juice must be shipped to the jelly plant, which defeats one of the economies in using concen-

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trates; there is no provision for volatile flavor recovery from the vacuum pan condensate; and by leaving pectin in the juice, clarification is often difficult; measuring and standardizing the pectin content is also rather difficult. Furthermore, a high pectin juice may gel during concentration. It is simpler for both the concentrate producer and the jelly manufacturer if all the pectin is removed from the juice during preparation; then the jelly maker can add the desired amount of pectin, knowing that the concentrate contains none. However, some juices may have so little pectin in them naturally that they can be clarified and concentrated easily without depectinizing. In this case the small amount of pectin in the concentrate can be neglected.

With a clear, pectin-free, full-flavored fruit juice concentrate as a starting material, it is fairly simple to process this into jelly on a continuous basis. The main requirement is to mix and dissolve the ingredients in the proper proportions, heating only enough to accomplish this and to pasteurize the jelly.

EXPERIMENTAL

Laboratory preparation of jelly. The best order of mixing the ingredients must be determined, as well as the amount of heating and mixing necessary to blend the ingredients into a uniform, high-quality jelly. The jellies made in this work consist of depectinized fruit juice concentrates, sucrose, invert sirup, fruit acid, pectin, and concentrated volatile flavor solution recovered from the juice concentration operation. There are many possible variations in mixing these ingredients, but certain principles based on theory and previous experience can be used to determine the most suitable combinations. The arrangement most used in this laboratory for preparation of jelly is shown in Figure 1.

The sirup made from dry sucrose was boiled to remove air bubbles which apparently were trapped around the sugar crystals. If the bubbles were not removed they carried through into the jelly as a scum, but if they were removed there was practically no scum. The sirup was in the range of 75 to 80% sugar. The sugar concentration in the sirup was varied depending on the degree of concentration of the fruit juice used. Invert sirup was added to the extent of 10 to 20% of the total sugar to preclude crystallization in the jelly or in the sirup when it was cooled.

Pectin was dissolved in water to make a 4% solution. The pectin must be dissolved in water or in a low concentration sugar solution to avoid precipitation. This was then added with stirring to the hot sirup in the feed flask and the mixture was reheated to about 190° to 200° F. in the small tubular heat exchanger. The pectin solution must be added to hot sirup; if it is added to a cold sirup the pectin is precipitated and further heating does not redissolve it well. This makes a poor jelly with a rather starchy taste.

The juice concentrate could be added anywhere in the process but has usually been added cold with the volatile flavor concentrate. This minimizes the heat damage and also makes possible the use of a juice concentrate which contains the returned volatile flavors rather than having the flavors packed separately. A 4- to 5-fold concentrate was used for much of the work reported here.

The acid was added near the end of the process, because adding acid earlier has been found to cause premature setting of the jelly *(3)*. Volatile flavors were added as near the end of the line as possible to prevent losses by evaporation. The juice and flavor concentrates, with the acid dissolved in them, could be added all together as a cold solution, because roughly 90% of the weight of the ingredients was in the hot sugar-pectin solution. Consequently, in the laboratory apparatus the temperature upon mixing did not drop below 180° F.

Pilot plant jelly preparation. In the pilot plant process the sirup was made separately so that it could be held hot for several hours if necessary. The pectin solution was metered in from a separate tank so that it would not be degraded by holding with the hot sirup. Thus in the pilot plant setup 3 metering pumps were used, one for the hot sirup, one for cold pectin solution, and one for cold juice concentrate containing returned volatile flavors and added acid. This arrangement is shown in Figure 2.

The sirup was circulated in a 50-gallon tank containing a steam coil by means of a centrifugal pump, P_1. This circulation served to maintain a uniform sirup temperature up to the metering pump, P_2. The temperature was held just under the boiling point by a thermocouple in the sirup line activating an electronic temperature controller on the steam line. Cold pectin and concentrate solutions were metered into the hot sirup by pumps P_3 and P_4. This would have caused a temperature drop to about 170° F. at the filler. Therefore, a 3-ft. steam jacket with atmospheric pressure steam was used on the line to the filler to bring the temperature of the jelly to 180° F. for filling. Since the temperature of the steam jacket was not high (less than the boiling point of the jelly mix) no burning occurred if assembly used was simple and no mechanical difficulties arose. the pumps were shut off. However, if the pumps were off more than about a minute the pipe containing the complete jelly mix had to be flushed out, because the jelly in it would begin to set.

The pilot plant described had a capacity of 600 lbs. of jelly per hour but was ordinarily operated at 300 lbs. per hour. The Figure 3 is a photograph of the pilot plant line. A typical formula for 100 lbs. of jelly is given in Table 1.

The metering pumps are calibrated and set according to the

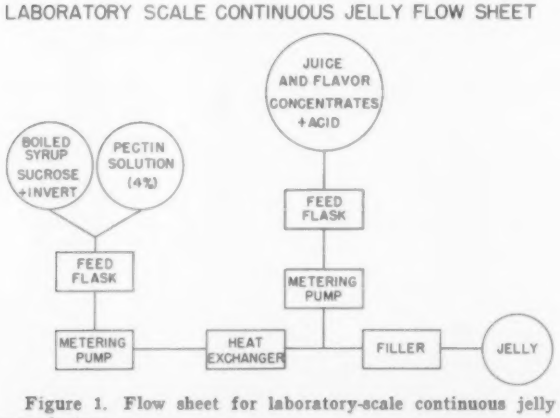

Figure 1. Flow sheet for laboratory-scale continuous jelly manufacture.

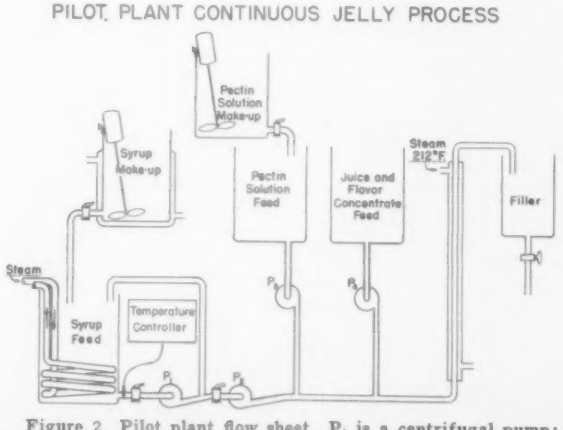

Figure 2. Pilot plant flow sheet. P_1 is a centrifugal pump; P_2, P_3, and P_4 are gear-type metering pumps.

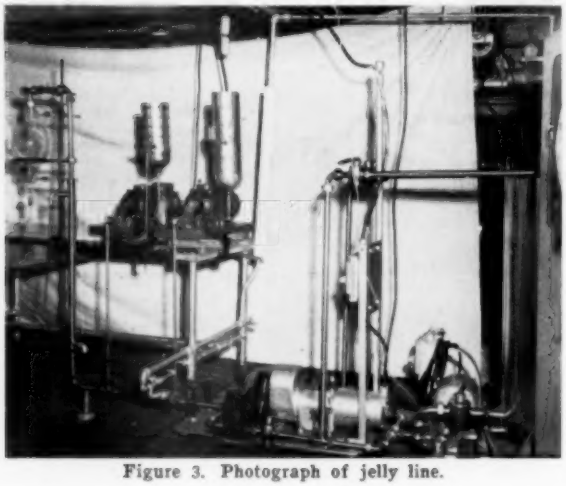

Figure 3. Photograph of jelly line.

formula and the flow rate desired. For example, at a rate of 300 lbs. of jelly per hour it takes 20 minutes to make 100 lbs.; therefore the weights of the 3 solutions given in the formula are divided by 20 to determine the delivery rate required for each pump in terms of lbs. per minute.

Whether acid is added in the formula depends on the pH of the fruit used. By regulating the pH of the jelly mix to about 3.1 the proper consistency was obtained with 0.4% pectin. If the pH of the mix without added acid was 3 or below, the pectin was decreased to 0.35%.

DISCUSSION

It is possible to concentrate juices more than is shown in the formula without lowering quality. This allows some leeway in formula variations. For instance, some single-strength juice could be added for flavor if a concentrate without returned volatile flavor was used. This method was used by MacDowell *et al.* *(7)* with orange juice concentrates and is similar to the system used in a batch process by Hoover, Nelson, and Steinberg *(5)* for making apple jelly. They divided the juice into 2 or 3 portions, and concentrated only one by boiling. The unboiled juice was added later to supply more fresh fruit flavor.

As mentioned earlier, one of the benefits in using concentrated fruit juice in a continuous process is to minimize discoloration due to heating. In the process described, there is virtually no heating of fruit material above 180° F.; consequently, there is no noticeable discoloration. Continuous-processed jellies had a much brighter color than available commercial samples. Jelly packed in No. 10 cans at 180° F. and air-cooled showed no brown discoloration despite the long cooling time. Evidently the rate of discoloration at 180° F. or below is very slow.

TABLE 1

Typical formula for strawberry jelly (for 100 lb. of 50/50 jelly)

Components	Amounts	Sugar equivalent
Syrup, 77° Brix (Contains 7.8 lbs. invert sugar)	78.3 lbs.	60.3 lbs.
Juice Concentrate, 41.9° Brix (Contains 0.12 lb. of 100-fold volatile flavor concentrate and 0.12 lb. citric acid)	11.7 lbs.[1]	4.8 lbs.
Pectin (4% Aqueous Solution)	10.0 lbs.	None
		65.1 lbs.

[1] Equivalent to 60.3 lbs. of 8° Brix juice.

SUMMARY

A process has been described for making jellies continuously from fruit juice concentrates. This process consists in mixing metered streams of sugar sirup, pectin solution and fruit juice concentrate. Only the sugar sirup is heated to a high temperature; therefore the fruit flavor and color are preserved by protection from heat damage. Consequently, jellies made by the continuous process described retain the quality of the fruit juice concentrate used.

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Simultaneous Determination of Total Count and Fluorescent Pseudomonads in Fresh Meat and Poultry

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THE NORMAL SPOILAGE of fresh meats, poultry, and fish is due primarily to the growth of *Pseudomonas* and *Achromobacter* genera, Stewart *(6)*, Haines *(2)*, Ayres *et al. (1)*, Jensen *(3)*. The identification of these organisms has, in the past, been based on cultural and morphological studies made on individual isolates. Since pseudomonads are so intimately associated with the spoilage of fresh meats, poultry, and fish, a method for their direct enumeration should be of great value in assessing the bacteriological condition of such products. This report describes a method whereby it is possible to determine simultaneously the total bacterial count and the numbers of pseudomonads. As will be indicated, the total counts obtained with the proposed method are significantly lower than are found by use of standard methodology; further, it is probable that not all members of the genus *Pseudomonas* are detected. But the limitations of the proposed total count procedure have been assessed by statistical analysis in terms of conventional methods, and it has been possible to show that there is a correlation between the psesudomonads detected and the organoleptic quality of the products studied.

MATERIALS AND METHODS

Direct determination of psesudomonads was accomplished by surface plating on the medium of King *et al. (4)*: proteose peptone 2%; glycerol 1%; K_2HPO_4 0.15%; $MgSO_4 \cdot 7H_2O$ 0.15%; agar 1.5%. The medium was autoclaved using conventional times and temperatures. Plates were poured with the sterilized and cooled agar. After solidification of the agar, the plates were placed in a 37° C. incubator over night in order to dry the surface of the agar. Surface plating was done by delivering 0.1 ml. of the appropriate sample dilution onto the surface of the agar. The inoculum was then spread evenly over the agar surface using a "hockey stick," a 3 mm. solid glass rod, 15 cm. long, bent downward at a 30° angle to give a foot 4 cm. long for spreading the inoculum. The "hockey sticks" were sterilized prior to use.

After appropriate incubation (3 days at 20° C.) total counts were obtained by counting colonies in the usual manner. An estimate of pseudomonads was made by examining the plate under ultraviolet light and counting fluorescent colonies. For this purpose, a portable 110 volt ultraviolet lamp was mounted in a box so that the plates could be examined beneath the light under conditions where outside light was excluded. It was found that colonies producing fluorescent pigment could be detected, even though such colonies might be outnumbered by non-fluorescent types 300 or 400 to 1. Pure isolates from fluorescent colonies were repeatedly shown to be gram negative motile rods with biochemical patterns characteristic of the genus *Pseudomonas*.

Conventional total counts were done using Tryptone Glucose Yeast Extract Agar, with incubation 3 days at 20° C.

EXPERIMENTAL

Comparison between Kings Medium (PG agar) and Tryptone Glucose Yeast Extract Agar (TGYE) for total count on fresh meats. Thirty-three samples of ground meat trimmings used during a storage study were plated on P.G. agar and on T.G.Y. agar. The total counts were transformed to logarithms and subjected to statistical analysis which revealed that the counts obtained by direct plating on P.G. agar were significantly lower than those obtained on T.G.Y. agar. The means were as follows:

	Logarithms	*Counts*
T.G.Y.	7.7393	5.4×10^7
P.G.	7.5247	3.35×10^7
Mean Difference	0.2146	1.64 Fold

The standard deviation of the difference between the media was 0.259 and the 95% probability limits for the difference in terms of logarithms were 0.2146 ± 0.5180 or -0.3034 to 0.7326. Transformed to total counts the limits were from -2.01 fold to +5.40 fold. Hence, the average we would expect to obtain on T.G.Y. would be 1.64 times as high as on P.G. medium, but individual counts might range from 2.01 times less to 5.40 times greater than those on P.G. medium.

Examination of the formula for P.G. agar (see Materials and Methods) indicates that the medium is nutritionally inferior to T.G.Y. agar. In an attempt to obtain counts more close to those obtained on T.G.Y. agar, the effect of adding 0.3% yeast extract and 0.2% glucose to the medium was tested, using 24 fresh meat samples. A statistical analysis of the logarithms of the total counts revealed that both P.G. Medium and modified P.G. Medium gave significantly lower counts than TGY and that the modification had not affected the P.G. Medium. The means were:

	Logarithms	*Counts*
T.G.Y.	6.0806	12.0×10^6
P.G.	5.9089	8.1×10^6
Modified P.G.	5.9582	9.1×10^6

It was later found that the lower counts obtained on P.G. agar could be traced to the method of sample analysis. A series consisting of 6 fresh meat samples were surface plated on P.G. Medium and on T.G.Y., and poured on T.G.Y. Statistical analysis of the counts showed that on T.G.Y. medium the surface plating technique gave significantly lower counts than pouring, and that the differences between T.G.Y. streaked plates and P.G. plates were too slight to be statistically significant. The means were:

	Logarithms	*Counts*
T.G.Y. Poured	9.3598	2.3×10^9
T.G.Y. Streaked	9.1417	1.4×10^9
P.G. (Streaked)	9.2017	1.6×10^9

Further, it was found that with fresh meats and poultry the major forms occurring grow with equal facility on both media.

From the foregoing it was concluded that P.G. agar may be used for determination of total numbers of bacteria in fresh meats, provided that the limitations of this method are taken in account in comparing results obtained to those with more standard media.

I. Pseudomonad development on stored ground meat

It has been our experience that whereas T.G.Y. and nutrient agars support luxuriant growth of pseudomonads, either in poured or streaked plates, only rarely does one find a strain which produces fluorescent pigment under these conditions. With P.G. medium, on the other hand, the production of fluorescin is enhanced and colonies producing the pigment may be detected readily under ultraviolet light, even though in many cases no fluorescence is evident in visible light.

In order to study the development of pseudomonads in relation to the organoleptic and bacterial quality of fresh meats, fresh beef and pork trimmings were coarsely ground and mixed in a plant scale chill mixer. Each product was then stored at 37° F. in boxes lined with waxed paper. Triplicate samples of each type of meat were removed initially and after storage 7, 10 and 14 days. Total counts and fluorescent colony counts were done on P.G. agar, as previously described. The trimmings were also evaluated organoleptically by a panel of 3–7 judges considered to be experts. The scale used by the judges ranged from 5 (fresh meat) to 1 (spoiled meat) for both odor and flavor. For example, a score of 1 was indicative of meat with an ammoniacal or putrid odor and a greening, browning or darkening of color.

RESULTS

The results of this experiment are summarized in Table 1. It will be noted that the pseudomonads constituted a negligible portion of the initial population but that with storage the fluorescent colonies constituted a significant part of the flora. Concomitant with this development, the organoleptic and general bacteriological quality deteriorated. At the end of the storage period, fluorescent colonies constituted 25–30% of the flora. We have observed a similar pattern repeatedly on different types and sources of fresh meats; the findings merely confirmed the reports of other workers previously cited. We have studied the nonfluorescent flora extensively and have found a number of genera involved, including *Achromobacter, Chromobacter, Streptococcus, Micrococcus,* and *Lactobacillus.* It has been our experience however, that the fluorescent pseudomonads are invariably found on stored fresh meats and that the organoleptic quality of the meat is closely associated with the numbers of these organisms present.

TABLE 1

Bacteriological and organoleptic changes in stored ground meat trimmings

Days storage 37° F.	Product	Bacteria per gram meat[1]		Quality evaluation	
		P.G.	Fluorescent colonies	Odor	Color
Initial	Beef	10,000	<1,000	5.0	5.0
	Pork	30,000	<1,000	5.0	5.0
7	Beef	150,000,000	35,000,000	3.8	3.3
	Pork	430,000,000	140,000,000	4.7	4.6
10	Beef	50,000,000,000	470,000,000	3.0	2.4
	Pork	2,800,000,000	500,000,000	4.3	4.6
14	Beef	9,100,000,000	2,300,000,000	1.8	2.6
	Pork	18,000,000,000	4,900,000,000	1.9	4.6

[1] Arithmetic average of three samples.
[2] Based on scale from 5 to 1, with value of 5 indicating sound fresh meat.

II. Development of pseudomonads on stored poultry

Young fryers were slaughtered, commercially processed, individually dry packed in cardboard trays, and wrapped in Saran. Each package was stored at 34° F. and contained 2 legs, 2 thighs, 2 wings, a split back and split breast.

Using the millipore filter technique of Silliker *et al. (5)*, duplicate surface samples were taken from the breast of each bird initially and after 2, 5, 7, and 14 days storage. In addition, approximately 1 square inch of kidney area was swabbed at the indicated sampling periods. Total counts and fluorescent colony counts were done as previously described, and the odor of the birds was graded. Duplicate birds were sampled at each period.

RESULTS

Results are summarized in Table 2. As with fresh meat, fluorescent pseudomonads form a significant portion of the total flora of poultry which has been stored under commercial conditions and concomitantly the general organoleptic and bacterial quality deteriorates.

DISCUSSION

Any investigator studying the bacteriology of fresh meat and poultry will inevitably encounter pseudomonad development. The method discussed above is a valuable tool in that it allows determination of these types quantitatively. This information combined with the total count becomes an excellent index of bacteriological condition. Moreover, in our study, P. G. Agar appears to support as many different kinds of bacteria as does Tryptone Glucose Yeast Extract Agar. Statistical analysis of total counts obtained from P.G. medium clearly established its limitations and makes it possible to correlate these counts with those obtained from conventional media. On this basis it seems reasonable to suggest that P. G. Agar could be profitably substituted for T.G.Y. Agar.

SUMMARY

A method for the simultaneous determination of total bacterial count and fluorescent pseudomonads is described. The development of fluorescent pseudomonads is found during storage of fresh meats and poultry, and an estimate of their numbers gives added information relative to bacteriological quality.

TABLE 2

Bacteriological and organoleptic changes in stored, packaged poultry

Days storage 34° F.	Bacteria per square inch of surface[1]				Odor[2]
	Total count		Fluorescent colonies		
	(Kidney)	(Breast)	(Kidney)	(Breast)	
Initial	250,000	200,000	8,000	<1,000	5.0
2	1,200,000	75,000	40,000	1,500	4.5
5	4,500,000,000	54,000	300,000,000	<1,000	4.8
7	6,000,000,000	10,000,000	50,000,000	10,000,000	3.3
14	57,000,000,000	260,000,000	39,000,000,000	180,000,000	1.5

[1] Arithmetic averages of duplicate samples from two different birds at each sampling period.
[2] Graded on a scale with 5.0 for excellent to 1.0 for repulsive.

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Influence of Re-using Chill Tank Slush Ice on Market Quality of Eviscerated Broilers[a]

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Water is an indispensable item in the poultry processing industry. It has been estimated that under commercial conditions 3 gallons of water are required to process a 3-pound broiler *(2)*. Considering that many poultry processing plants today process several thousands of birds per hour, a large volume of water is necessary for daily operations. A significant portion of the water used in the processing operation serves to remove body heat from the eviscerated carcasses. This is usually accomplished at the end of the processing line by submerging the carcasses in a mixture of crushed ice and water which is commonly referred to as slush ice chilling.

The poultry processing industry, as many other water consuming industries, is beginning to realize the necessity of conserving water in order for existing supplies to be sufficient for an expanding market. Consequently, some plants have resorted to the use of chill tank water a second time for cooling carcasses. The effects of re-using chill tank slush ice on the market quality of eviscerated broilers have not been investigated. The purpose of this study was to determine the influence of re-using chill tank water and ice on bacterial numbers and shelf life of eviscerated broiler carcasses.

EXPERIMENTAL

All carcasses used in this study weighed approximately 2½ pounds and were secured from a commercial poultry processing plant in Raleigh, North Carolina. Approximate weights were determined on the processing line by an automatic weighing device. Carcasses were packed ice free in wire-bound, wooden boxes and transported (approximately 20 minutes) to the College Poultry Science Laboratories for treatment.

The variability in bacterial numbers on the skin of carcasses prior to treatment was determined. Four groups consisting of 10 carcasses per group were analyzed for bacterial numbers as they were removed from the processing line at 2-hour intervals. This procedure was replicated on 2 different days and the data of the 2 studies were combined in the statistical analysis.

Slush ice for chilling carcasses was prepared by placing 11 gallons of distilled water and ice (9 water:2 ice) in a 20 x 27 in. sanitized, cylindrical, galvanized chill tank. The level of the slush ice was marked on the inside of the container. Samples of the water and ice mixture were analyzed for bacterial numbers and per cent light transmission. Per cent light transmission was used as an objective determination of chill tank water turbidity. The chill tank was placed in a 35° F. walk-in refrigerator in order to limit excessive melting of the ice.

Three groups of carcasses were removed from the processing line at 1-hour intervals. The first group consisted of 30 carcasses and the subsequent 2 groups of 20 carcasses each. In the initial group 10 carcasses were analyzed for skin bacterial numbers after removal from the processing line. The remaining 20 carcasses were placed in the slush ice and held therein without agitation for 1 hour. After the holding period, the carcasses were removed from the chill tank and samples of the chill water were analyzed for bacterial numbers and per cent light transmission. Ten carcasses were placed in polyethylene bags and held at 35° F. for shelf life determination. The remaining 10 carcasses were analyzed for bacterial numbers. The determinations of carcass shelf life, carcass bacterial numbers and bacteria per ml. of slush ice were made according to the methods of Fromm *(1)*. The per cent light transmission of the chill tank water was determined in a Coleman Spectrophotometer Model 14 set at 450 mμ.

Immediately after the first group of carcasses had been removed from the chill tank, the water in the tank was brought up to its original level by the addition of distilled water and ice. A second group of 20 carcasses that had been removed

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from the processing line within the previous 20 minutes was added to the chill tank and held therein for 1 hour. Identical analyses were made with the second as with the first group. The slush ice was again brought up to its original level and a third group of carcasses was introduced into the chill tank. Identical procedures were followed and analyses made with the third group of carcasses as with the first and second groups.

This study was designated as Experiment A. The study was replicated and the bacterial counts pooled in the statistical analysis.

In order to explore further the influence of re-using chill tank slush ice on eviscerated broiler carcass quality, a second study designated as Experiment B was undertaken whereby slush ice was used to cool 5 successive groups of carcasses in the same chill tank. Analyses and procedures were identical with those in Experiment A except that the carcasses and slush ice were analyzed after the first, third and fifth groups had been cooled in the chill tank. This study was also replicated and the data pooled in the statistical analysis. Further re-use of the chill tank slush ice was limited due to the length of the work day at the poultry processing plant and the corresponding supply of carcasses.

Five carcasses used for bacteriological analysis within each treatment of Experiments A and B were cooked and tasted to determine if the re-use of chill tank slush ice had any effect on the odor and flavor of the carcasses. The carcasses were wrapped in aluminum foil and baked at 280° F. until the temperature of the pectoral muscles reached 180° F. The carcasses were permitted to cool at room temperature and different parts of each carcass were sampled by a panel of 5 during each tasting session. Taste was analyzed on a comparative basis so that only the presence or absence of off flavor was noted.

RESULTS

The variability in skin bacterial numbers per square inch of carcasses removed from the end of the processing line is shown in Table 1. No differences were found in bacterial numbers between groups.

The bacterial numbers and turbidity of the chill tank water increased with each successive addition of carcasses (Figure 1). The level of the mean bacterial numbers per ml. of chill water was higher in Experiment A than in Experiment B (Figure 1). Since the trend lines essentially parallel each other, the difference in magnitude of bacterial numbers in the slush ice may be attributed to day-to-day variation in bacterial load on the carcasses.

The influence of repeated use of chill tank slush ice on bacterial numbers per square inch of broiler carcass is shown in Figure 2. Slush ice chilling caused a reduction in carcass bacterial numbers. This decrease in bacterial numbers was apparently caused by the washing effect of the slush ice as evidenced by the increase in the numbers of bacteria per ml. of chill tank water. The re-use of the slush ice caused a slight upward trend in the numbers of bacteria per square inch of carcass skin. Bacterial numbers per square inch of carcass skin were consistently less in Experiment A than in Experiment B. The difference may be attributed to the variation in carcass bacterial load between days of production. The statistical analysis showed that there were significant differences between replications within Experiment A and Experiment B. There were no differences in carcass bacterial numbers between treatments in both Experiments A and B. The coefficients of variation for bacterial numbers of carcasses in Experiment A and B were 61% and 82%, respectively.

TABLE 1

Mean bacterial numbers per square inch of carcass skin from groups of carcasses removed from the end of the processing line at 2-hour intervals on 2 different days[1, 2]

Group	Group mean bacterial numbers[3] per square inch of skin × 10^4	
	Day 1	Day 2
1	47	31
2	54	64
3	40	53
4	46	54

[1] Each observation represents a geometric mean for 10 carcasses.
[2] No significant difference between groups.
[3] Coefficient of variation—68% on original counts and 13% on group mean counts.

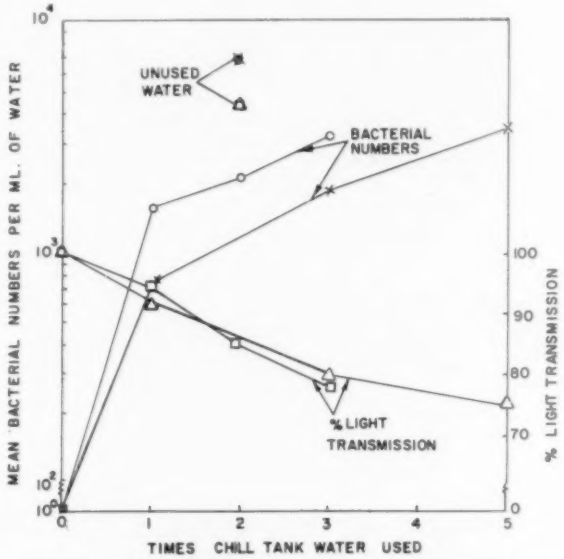

Figure 1. The influence of re-using chill tank slush ice on turbidity and mean bacterial numbers per ml. of slush ice (chill water).[1]

1. Experiment A ○——○ Bacterial numbers
   Experiment B ×——× Bacterial numbers
   Experiment A □——□ Turbidity
   Experiment B △——△ Turbidity

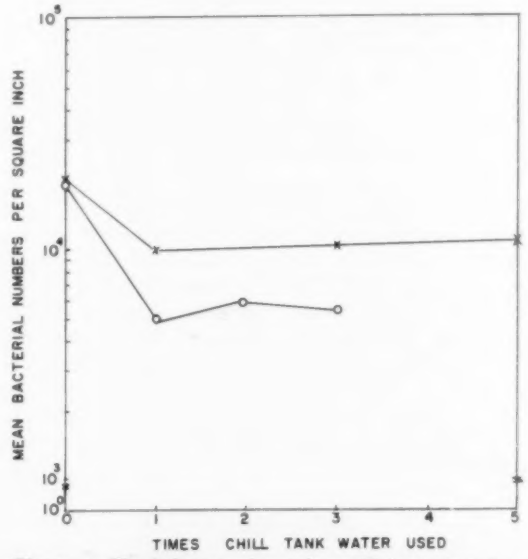

Figure 2. The influence of re-using chill tank slush ice on mean bacterial numbers per square inch (skin) of chilled carcass.[1]

1. Experiment A ○——○
   Experiment B ×——×

The influence of re-using chill tank slush ice on the shelf life of broiler carcasses is shown in Table 2. The number of times that the slush ice was used had no effect on the shelf life of the carcasses.

TABLE 2

Influence of re-using chill tank slush ice on shelf life of eviscerated broilers[1]

Experiment	Trial	Times chill tank slush ice used				
		1	2	3	4	5
		Shelf life (days)[2]				
A	1	11.7	10.8		11.6	
	2	10.5	11.0		11.0	
B	1	11.5			11.3	11.7
	2	11.7			11.7	11.8

[1] Each observation represents an arithmetic mean for 10 carcasses.
[2] No significant differences between means.

The evaluation of the carcasses for off-flavor or off-odor did not reveal any evidence that the re-use of chill tank slush ice imparted any taint to the flavor of the chicken flesh or to the cooking aroma.

DISCUSSION

Each successive re-use of chill tank slush ice resulted in the cooling medium becoming more turbid and reddish in appearance. These results indicate that the market quality of the eviscerated broiler is not governed by the color of the chill tank slush ice. The number of microorganisms on a carcass as it is removed from the chill tank may be indicative of the market quality and shelf life of that carcass *(1)*. The discoloration of the slush ice is apparently caused by the accumulation of carcass fluids (blood and cell sera) in the slush ice.

If the re-use of the chill tank slush ice were to cause a drop in carcass quality, it would be inferred that the slush ice is depositing more bacteria than it washes off. In this study this condition did not occur (Figures 1 and 2).

It has been shown that under the conditions of this study the re-use of chill tanks slush ice was not detrimental to product quality. Under commercial conditions, the governing factors that would probably limit the re-use of chill tank slush ice would be (1) the bacterial load in the water and ice (2) bacterial numbers on the chill tanks and (3) the degree of bacterial contamination of the carcasses as they are removed from the processing line. Excessive contamination from any one of these factors could be cause for a shortened carcass shelf life and an inferior product regardless of how many times the chill tank slush ice is used.

SUMMARY

The influence of re-using chill tank slush ice on eviscerated broiler carcass quality was investigated. Under the conditions of this study the re-use of chill tank slush ice as many as 5 times did not significantly influence the bacterial numbers on chilled carcasses nor affect the shelf life of the carcasses or flavor of the cooked product.

Acknowledgments

The author wishes to expess his appreciation to Dr. R. J. Monroe for aid in conducting the statistical analysis and to Mr. E. T. Watson of Watson's Seafood and Poultry Company, Raleigh, North Carolina, whose cooperation made this study possible.

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Notes and Letters

Problems in Determining Fish Freshness

IT IS UNIVERSALLY RECOGNIZED that development of some kind of scientific test or tests that would accurately and consistently indicate objectively the freshness of fish and possibly also predict keeping quality would be of great value to the commercial fishing industry. A number of chemical freshness tests have been proposed, but none of them are consistently accurate in measuring fish freshness for all species.

In this report, an attempt is made to describe just what the problem is that must be solved before satisfactory tests for the freshness of fish can be evolved. The report includes a consideration of precisely what we are trying to measure when we attempt to develop a test for the freshness of fish.

WHAT CAN FISH FRESHNESS TESTS MEASURE?

Time since fish was caught. A number of quite distinct objectives may be considered as being the function of a fish freshness test. One is to determine the elapsed time since the fish were caught regardless of whether any alteration in the fish has occurred. Some of the purposes of measuring this elapsed time are (1) to aid in the enforcement of fishing regulations by showing whether a batch of fish could have been caught before the fishing season opened and (2) to give a supplementary indication of probable future keeping quality.

Such an estimate may be almost impossible to make because any determination of elapsed time since the fish are caught must depend on some measurable change in the fish. Such changes are a function not only of time but also of other factors, particularly the temperature at which the fish has been held. We might then restate this first objective of fish freshness tests as being the determination of the time since the fish was caught, as a function of storage temperature, but without regard to major alterations in the physical or chemical condition of the fish.

Alteration from original condition. The most usual objective of a fish freshness test is the measurement of actual changes that have occurred in the fish since catching, with particular reference to those changes that are considered to be of a detrimental nature. Such changes may encompass a wide range from changes in appearance of the whole fish to chemical changes that result in adverse texture or in development of off odors and flavors. Most of the chemical tests so far proposed would seem to be aimed at this objective.

Keeping quality. A third objective of fish freshness tests is to measure the future keeping quality of the fish quite regardless of the exact condition of the fish at the time of sampling. Keeping quality can be measured in one of two general ways.

Estimation based on several tests. It is possible, by comparing results of several entirely different types of tests, to obtain some measure of keeping quality. Griffiths and Stansby *(1)*, for example, suggested that, by considering both chemical tests and total bacterial count, a measure of keeping quality could be obtained. Fish of equal freshness as measured by a chemical test, but of differing bacterial counts, would have different keeping quality based upon the bacterial contamination. In this connection, it might be mentioned that it is not the *total* count that is really critical, but rather the number of *spoilage* bacteria present. When total counts are used (because they are easier to carry out) correlation with keeping quality is obtained only insofar as some degree of proportionality exists between total count and count of spoilage bacteria.

Estimation based on accelerated tests. It has been customary to estimate keeping quality of oils by subjecting them to accelerate storage conditions and determining how long a time is required for them to reach a stipulated degree of rancidity as, for example, when they are stored at an elevated temperature with aeration. Similar accelerated tests have been used less frequently for determining bacterial decomposition by measuring the time required at elevated temperature for the sample to reach a stipulated stage of decomposition.

CHANGES OCCURRING DURING DETERIORATION OF FISH

Fundamental changes. Changes that occur when fish deteriorate can be looked upon as being of 2 types. One of these is what may be termed a fundamental change, and tests used to measure such changes can be termed fundamental tests *(2)*. The fundamental tests are those which measure factors that contribute to the quality of fish. These may be based upon changes that result directly in alteration of texture, flavor, odor, or appearance; or they may be changes that alter the keeping quality. Examples of the latter include development of lactic acid in the flesh, at low enough concentrations not to affect the flavor, yet in sufficient quantity to lower the pH enough to retard bacterial action; and presence of large bacterial loads, which although not apparent organoleptically, would increase spoilage rate.

Accessory changes. The second type of change is accessory changes, and accessory tests are used to measure them. Accessory changes include those that occur in deteriorating fish without directly affecting quality.

Changes in the appearance of the eyes of fish is an example of an accessory change. The well known changes in the appearance of fish eyes, such as the development of cloudiness, generally run parallel to other undesirable changes in the flesh. Since, however, the eyes are not eaten, any change in them has no direct bearing on quality. Another type of accessory change involves the formation of chemical substances in the flesh of the fish that do not in any way alter flavor, odor, texture, or appearance. Such changes—for example, formation of carbon dioxide—may be used as an accessory test for the quality of the fish if it occurs parallel with deterioration directly causing the adverse changes.

Some changes that appear to be of a fundamental nature may be, at least partially, of the accessory type. An example of this is the formation of trimethylamine. A test for trimethylamine is often used as a measure of freshness for certain species of fish. In a general way, quantities of trimethylamine measured as milligrams of trimethylamine nitrogen per 100 grams of fish correspond to the degree of freshness approximately as follows: 0.0 to 0.4, fresh; 0.4 to 2.0, incipient decomposition; 2.0 to 4.0, stale; and 4.0 to 10, putrid.

Trimethylamine possesses an odor similar to that of decomposing fish. The assumption has been held that trimethylamine in the quantities indicated above impart to the stale or spoiled fish its unpleasant odor. Some fishery products may contain many times these amounts of trimethylamine, however, without possessing any off odor whatever. The author, for example, has examined lots of canned salmon that had been stored in the can for several years and that contained over 100 mg. of trimethylamine nitrogen per 100 g. of fish; yet there was no fishy or stale odor present at all. Conversely, if trimethylamine is added to and mixed with fresh fish to give a concentration of

10 mg. of trimethylamine nitrogen per 100 g., no fishy or stale odor is observed. These facts would indicate that although a test for trimethylamine in fish may give a measure of its freshness, this correlation is due not entirely to the fact that the odor of stale fish is caused primarily by trimethylamine. Rather, the breakdown of trimethylamine oxide to trimethylamine happens to parallel the spoilage of fish, and because of this parallel reaction, a measure of one gives us a measure of the other.

SPOILAGE PATTERNS

Even when dealing with a single species of fish, we find that many different types of changes can occur as fish spoil. Several entirely different types of reactions are involved in these changes. Oxidation of flavor constituents may develop; bacterial spoilage may occur; or enzymatic changes may take place. These different types of reactions result in such changes in the fish as loss of flavor, development of off odors and off flavors, alteration in texture, and diminishment of keeping quality.

With so many different changes occurring, it is not surprising that the spoilage pattern varies widely from one batch of fish to another. The pattern also is influenced by the many causative factors influencing the different reactions. Rigor mortis changes, for example, are governed in part by the method used to catch the fish (owing to different degrees of struggling of the fish in the nets) and also by the rapidity with which the fish are iced after being caught. This treatment of the fish, in turn, is reflected by the quantity of lactic acid accumulating in the fish and the length of time that the accompanying lowered pH persists in the tissue (resulting in retarded bacterial action). Another example of the various factors influencing the spoilage pattern is the different spoilage bacterial flora that occur in different lots of fish. Still another example is furnished by the differences in enzymatic action emanating from the visceral tract, since this action is greater or less, depending upon the quantity and the type of feed in the fish.

With all of these reactions and factors varying in widespread manner, the pattern of spoilage from one lot of fish to another also varies widely. In some lots of fish, one type of odor or flavor develops at an early stage and predominates. In another lot, this same odor or flavor may not develop until late in the spoilage—or not at all—and some other changes may occur instead.

MEANINGLESSNESS OF SINGLE FRESHNESS TEST

The opinion often has been expressed that our fishery technological research should be directed toward developing a single objective freshness test that will work for all species and for all batches of fish. From what has been said above, it is apparent that the development of a single reliable test of freshness is just as impossible as it would be to expect that chemical analysis of the exhaust fumes from an automobile would give a complete diagnosis of the state of the vehicle, including condition of tires and battery.

Since we are dealing with many entirely different reactions resulting from different causes and leading to entirely different changes in the fish, it is obvious that a number of entirely different tests must be applied to detect these changes. Thus, we should not compare chemical test A with chemical test B and say that one is better than the other as a freshness test. Rather, we should determine which chemical or other tests, when run in conjunction with one another, will give us the best overall criteria as to the deterioration that has taken place.

When looked at from this point of view, the situation is quite different from that in which one test is compared with another in furtherance of the idea that the tests are mutually exclusive and that only one is to be used. Thus, a group of 3 chemical tests, none of which gives good correlation with freshness for all lots of fish, might work well as a unit if applied together.

FUTURE RESEARCH

Since freshness of fish is not a simple quantity that can be measured by a single test, future research on freshness tests should follow a more logical pattern based on development of tests for certain quite definite aspects of freshness. At least 5 different aspects need to be considered: (1) chemical changes that result in loss of normal flavors, (2) chemical changes that result in development of off flavors and odors, (3) changes that result in texture alteration, (4) changes in keeping quality, and (5) chemical changes that might correlate with the length of time since the fish was caught.

Loss of normal flavors. Fresh fish is characterized by a pleasing flavor characteristic for the particular species. This flavor diminishes soon after the fish is caught, and this loss in flavor is just as much a loss in freshness as is the development of the usual spoilage odors and flavors. Any complete examination for freshness of fish should take such changes into account.

Unfortunately, our knowledge of the chemical compounds giving the normal flavors to fish is almost nonexistent. Long-term research is needed to characterize these compounds. When this knowledge becomes available, tests probably could be developed to follow the losses in these flavor components.

Development of off flavors and odors. Research is needed to correlate a number of long-existing tests and of newly devised ones for fish-decomposition products (which are related to off odors and flavors) with the various patterns that develop as fish spoil. Most past research has dealt with only one test at a time. A coordination of the findings, using different tests, might lead to important discoveries.

Texture. The texture of fish alters during spoilage. Physical or other tests are needed to follow such changes. Texture, like other freshness criteria, is not a simple term but consists of many component factors each of which needs to be tested in a different way. New and better ways of measuring texture components such as toughness, moistness, and drip are needed.

Keeping quality. Research is needed to develop methods for estimating the keeping quality of fish. Investigations along at least two lines might be profitable. Identification of the kind of bacterial flora and estimation of the quantity of bacterial flora causing

spoilage would be one approach. Development of accelerated spoilage tests would be another.

Time since fish was caught. One objective of fish freshness tests was mentioned earlier as being an estimate of the time since the fish was caught. It was pointed out that this time is also related to storage temperature. Accumulation of any given chemical substance causing spoilage odors and flavors cannot be expected to correlate with this time-temperature relationship because as we have seen, the spoilage pattern varies so greatly from one lot of fish to another.

Accessory tests have been mentioned as being those that measure the formation of substances not causing spoilage odors yet developing parallel with spoilage. It is possible that some changes in fish muscle which start to occur shortly after the death of the fish might be found which correlate with the time-temperature relationship.

Certain chemical compounds develop as a result of muscle metabolic changes that are reversible during life. After the death of the fish, these substances accumulate. Research along these lines might lead to development of a test to ascertain the time since the fish was caught. Other similar possibilities exist in connection with early changes in such physical properties as electrical conductivity or dielectric constant of fish muscle or such physiological properties as stimulation to application of electrical shock to the muscle.

Research on early changes in fish before the onset of extensive spoilage also offers a promising approach to estimation of keeping quality, since a comparison of the time-temperature status of the fish with other spoilage indices would enable an estimation to be made as to keeping quality. Thus a sample showing that considerable time had elapsed since catching, yet showing no appreciable accumulation of odoriferous products, would probably have a much shorter storage life than would one showing that only a very short time had elapsed.

Simplified procedures for freshness tests. For application to most commercial problems, a relatively simple test is needed that can be carried out in a period of minutes or, at most, in a very few hours. This requirement is especially the case where purchase of a lot of fish is dependent upon the results of the test. Many of the presently available freshness tests are too time consuming for such use. Hence, research is needed on tests that may be ultimately simplified for use in the field, where the elapsed time required to run the test is of paramount importance.

SUMMARY

Spoilage of fish is a complex process that can proceed along many diverse lines. The pattern followed is dependent upon various factors many of which are correlated with handling methods. Even different batches of the same species of fish will spoil in quite different ways.

No one freshness test possibly can give a good measure of the relative freshness of fish. Any one freshness test should be patterned toward some specific objective such as (a) measurement of loss of normal flavor, (b) measurement of development of off flavor and odor, (c) estimation of keeping quality, or (d) estimation of time since the fish were caught.

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September 30, 1957.

Effect of Selected Antibiotics on *Pseudomonas fluorescens, Pseudomonas fragi,* and *Pseudomonas putrefaciens* [a]

THE INHIBITORY EFFECT of antibiotics on microorganisms has been of great interest to the dairy industry mainly because of *(a)* the effect of residual antibiotics in milk from treated mastitic udders on the activity of lactic acid bacteria and *(b)* the possible use of these agents in improving the keeping quality of milk in areas where present accepted methods of production and handling are not yet feasible. In the present study the effect of 9 antibiotics on various pseudomonads was determined.

The 7 cultures of *P. fluorescens*, and the 10 cultures of *P. fragi* and *P. putrefaciens* were grown on slants of Plate Count agar *(1)*. The growth which developed after 24 hours at 25° C. was washed once with ice cold phosphate buffer (0.0005*M*, pH 7.0) collected by centrifugation and re-suspended in buffer. The tests were set up according to the common two-fold serial dilution technique. The final volume in each tube was 1 ml. and consisted of 0.5 ml. of diluted culture (10^5 cells/ml.) and 0.5 ml. of a mixture of antibiotic and growth medium. The concentration of the antibiotics in the series ranged from 0.39 to 100 μg. per ml. The tubes then were incubated at 25° C. for 5 days. Growth in nutrient broth was assessed on the basis of turbidity; in litmus milk on the basis of physical changes in comparison with corresponding controls. The lowest concentration of antibiotic which prevented growth under the conditions outlined above was considered the minimum inhibitory concentration (MIC). A loopful (0.01 ml.) of the test media in the series then was transferred on Plate Count agar. The plates were incubated for 5 days at 25° C. The lowest concentration of antibiotic which prevented subsequent growth on Plate Count agar was considered the minimum lethal concentration (MLC).

The data (Figures 1, 2, 3) indicate that penicillin had no inhibitory effect on any of the test species. A similar result was observed for carbomycin and bacitracin on *P. fragi* and *P. fluorescens*. The majority of the test cultures were more sensitive to oxytetracycline, chlorotetracycline and tetracycline than to the other antibiotics. Within the limits of the concentrations studied chloramphenicol and polymyxin B were more inhibitory to *P. putrefaciens* than to *P. fragi* and *P. fluorescens*. At the concentrations studied chloramphenicol did not inhibit growth of *P. fluorescens*. Different strains of the same species showed great differences in the sensitivity to the various antibiotics. The data also indicate that the MIC of the antibiotics for the various strains of the test species were considerably higher in milk than in nutrient broth. Price *et al. (2)* observed a similar phenomenon with mastitis organisms. They also showed that $CaCl_2$ added to nutrient broth at the level of milk raised the inhibitory levels of neomycin, dihydro-

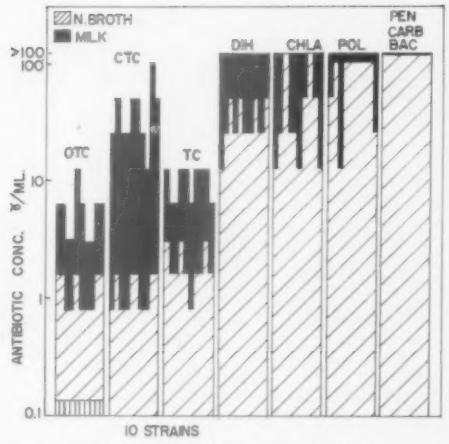

Figure 1. Minimum inhibitory concentrations of nine antibiotics for ten strains of P. fragi (oxytetracycline = otc, chlorotetracycline = ctc, tetracycline = tc, dihydrostreptomycin = dih, chloramphenicol = chla, polymyxin B = pol, penicillin = pen, carbomycin = carb, bacitracin = bac).

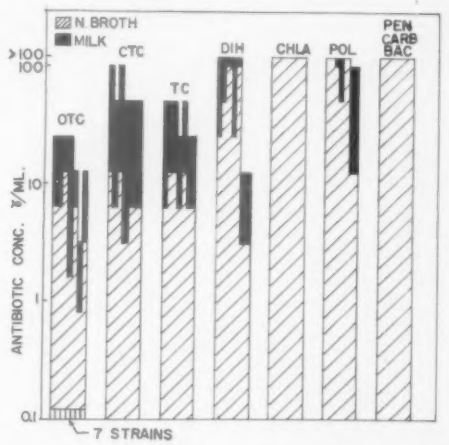

Figure 2. Minimum inhibitory concentrations of nine antibiotics for seven strains of P. fluorescens.

[a] Journal Paper No. 2717 of the Texas Agricultural Experiment Station, College Station.

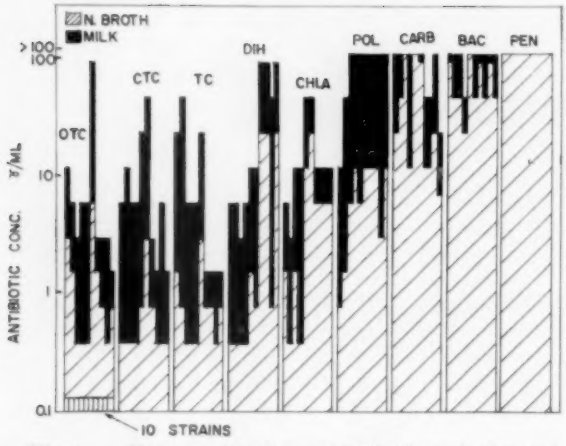

Figure 3. Minimum inhibitory concentrations of nine antibiotics for ten strains of P. putrefaciens.

streptomycin, chlorotetracycline, oxytetracycline, and tetracycline. No appreciable loss however in antibiotic activity in this medium with Ca could be detected by the diffusion plate assay.

No estimation of the MLC of penicillin, carbomycin, bacitracin, chloramphenicol, polymyxin, and dihydrostreptomycin could be obtained since they exceeded the highest level tested (100 μg./ml.). Of the other antibiotics an average of about 16 times the concentration needed for inhibition in nutrient broth was required to produce a lethal effect. The MLC in milk in most instances exceeded the highest concentration of antibiotic tested (100 μg./ml.).

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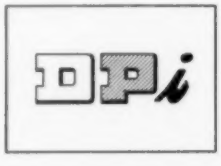

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CHICAGO

The Chicago Chapter was the guest of the Corn Products Refining Company for the March meeting. The meeting consisted of a guided tour of the plant facilities and a very delightful dinner at Mangam's Chateau. After the dinner, Dr. Albert L. Elder, Director of Research, Corn Products Refining Company, spoke about the various procedures used in the wet milling industry and discussed some of the research problems encountered as well as some of the benefits which have been reaped through the application of research to the solution of technical problems. The meeting was an outstanding success.

—H. P. Furgal

INDIANA

The Indiana Section members attending our March meeting enjoyed a movie on "Careers in Bacteriology" and a paper on the needs for research in the area of food microbiology given by Dr. L. S. McClung, Head of the Department of Bacteriology at Indiana University.

Section members were particularly interested in the production of the career movie as presentation of this nature may introduce potential food technology students to our profession. Dr. McClung discussed the problems and expenses involved in producing such a movie. He pointed out that a career movie gives a more wide distribution of information and a more complete discussion of materials than individual addresses.

A social hour and chicken dinner preceded the meeting.

MINNESOTA

The March meeting of the Minnesota Section of The Institute of Food Technologists featured Dr. L. E. Clifcorn, Supervisor, Container Utilization, American Can Company, Barrington, Illinois as guest speaker.

Dr. Clifcorn, who has been active for many years in the field of chemical contaminants of food and who aided in the formulation of the Miller Bill on Pesticides, addressed the group on the subject of "Chemical Additives—The Problem."

Following a brief review of the early history of the problem, the speaker noted that existing Food and Drug legislation broadly covers "poisonous and deleterious substances" in foods but does not adequately define such substances.

It was estimated that it would cost an industry from $50,000 to $100,000 and possibly as high as $250,000 to adequately prove the safety of a new chemical additive. Such costs would be prohibitive and would discourage research, according to Dr. Clifcorn.

"Packaging materials," said the speaker, "are, or can be a source of undesirable contaminants." Describing work done several years ago when enamels were being tested for use on interiors of metal cans destined to contain foods, Dr. Clifcorn said that a total of 24 different enamels were subjected to physiological tests by the American Can Company and

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It would be interesting to experiment with other ratios of triglyceride to monoglyceride. This is for food processors to do, not for us. Our business is making Myverol® Distilled Monoglycerides so pure they can give this effect. We shall be delighted to supply food processing firms with free samples for their experiments. ***Distillation Products Industries,*** Rochester 3, N. Y. Sales offices: New York, Chicago, and Memphis • W. M. Gillies, Inc., West Coast • Charles Albert Smith Limited, Montreal and Toronto.

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chemical tests by the Continental Can Company. The results from both tests were approved by the Food and Drug Administration.

The speaker felt that packaging materials should be classed by themselves as compared to additives, and that the use of the term "toxicity" when referring to packaging materials is undesirable since it gives the impression that a material is harmful. He suggested that terms such as "safety" and similar positive terms be used in order to avoid giving the impression that a substance is harmful at any level of intake.

A Food Additive, according to Dr. Clifcorn, should not be used for a questionable operation if the same results can be obtained by good operational practice. If such a substance is used, both Industry and the Food and Drug Administration should pass on whether such a chemical substance is safe, or not safe.

The argument that a chemical substance is of functional value should be answered in terms of the "question of safety for use under the conditions of use."

MOHAWK VALLEY

Thursday evening, March 20, the Mohawk Valley Regional Section, Institute of Food Technologists met in the personnel department at Beech-Nut Life Savers, Canajoharie, New York.

The principal speaker for the evening was Mr. Barton H. Siebers, Assistant Head of Research of the Nestle Company, Fulton, New York. Mr. Siebers gave a talk on "Quality Control Applied to the Manufacture of Chocolate Products." He also talked briefly about his company's history, and the responsibilities and functions of his department. Following his talk, Mr. Michael DeBenedictis, Assistant Head of Quality Control, The Nestle Company, showed a color movie "The Chocolate Tree."

NEW YORK

At the March 19th meeting of the New York Section at the Brass Rail Restaurant, Dr. Bernard L. Oser presented an address on " Food Additives." Dr. Oser, President and Director of Food and Drug Research Laboratories, Inc., has an extensive background in the field of food and drug regulation and legislation, and his remarks provided an interesting picture of the status and reasons for present and proposed food and drug regulation.

Dr. Oser reported that more countries today are concerned with food and drug matters than with control of the atom bomb. This interest reflects the "enormous amount of confusion and uncertainty" throughout the world of food processing and food distribution on this vital topic. World War II produced incentives for the development of a number of synthetic chemicals which today are among the additives for food processing and manufacture. Chief among these chemicals, and the one of greatest concern at this time, are the pesticides, which helped the allies produce greater yields in agriculture. The war also created the need to protect and preserve foods for the armed forces, from which arose the extended use of antioxidants and preservatives. The packaging industry has also had a tremendous technical growth since World War II, with the safety of packaging materials becoming a matter of food and drug concern.

As the use of food additives grew, interest in their control spread downward from federal to state and local levels, Dr. Oser pointed out. In addition, there have been repercussions in foreign countries as a result of technical developments in food additives. Policies vary widely, from the liberal approach of permitting that which is not specifically prohibited to the conservative one of prohibiting anything not specifically permitted. There are also wide variations in enforcement policies, the U. S. having perhaps the best enforcement of any country.

Dr. Oser also pointed out that policies concerning permissible food additives very often reflect local needs. For example, while the U. S. permits the use of certain antibiotics in poultry only, Canada, having an important fisheries industry, permits the antibiotic treatment of fish as well as poultry. India, lacking refrigeration and speedy transportation, also permits the use of antibiotics in fish preservation to insure the preservation of an important source of protein in the national diet.

While there may be good reasons for variations in national policies, Dr. Oser emphasized that the establishment of a number of conflicting codes among the states or cities of the U. S. could lead only to chaos for the food preserver and manufacturer. With this fact in mind, he discussed briefly the present status of proposed legislation and regulation, particularly that of the Federal, New York State and New York City governments.

Food Technologists Needed for Foreign Service. The Chapter received greetings recently from Tehran, Iran, sent by Dr. T. L. Swenson, U. S. Operations Mission, now acting as Chief Advisor to Ministry of Commerce of Iran on the establishment of "Quality and Performance Trade Standards." Dr. Swenson reports that the U. S. Foreign Aid Program needs more well-trained, mature food technologists. "Spread the gospel. Foreign service is very interesting," he writes.

Pesticides and Food Processing. The February meeting featured a talk on "Pesticides and Food Processing" by Dr. Wendel F. Phillips, Beech-Nut Life Savers, Inc. Dr. Phillips stressed that the food processor's problems are individual, depending on his product and his consumer, and that it is necessary for each manufacturer to appraise the various factors involved to determine exactly what his problem is, and, in fact, whether or not he really has a problem.

One common problem facing the food industry in general is compliance with the tolerances established under the regulations set forth in the Miller Bill, but, he added, the law provides protection for all—procesor as well as consumer. On the other hand, the necessity, usefulness and advantage of pesticides are established, for without them, we certainly would not have our present high standard of living nor enjoy the top quality foods we eat.

In discussing insecticides, Dr. Phillips mentioned that they have been used widely for the past 15 years and all indications are that when used in accordance

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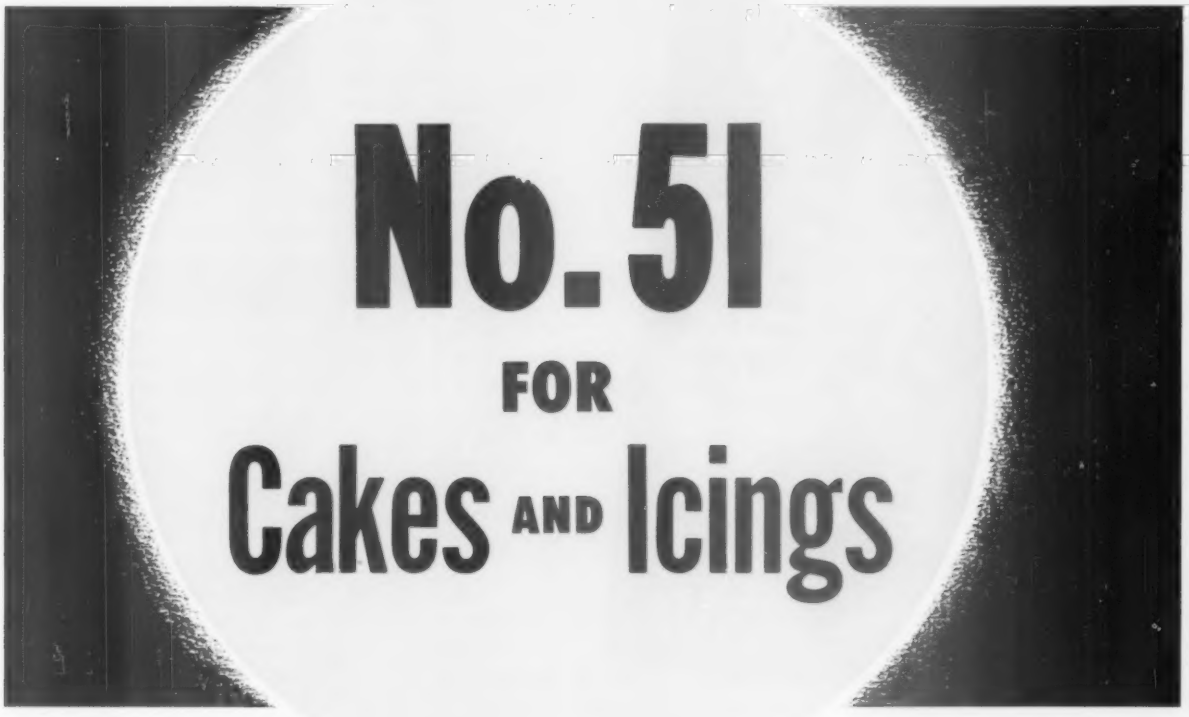

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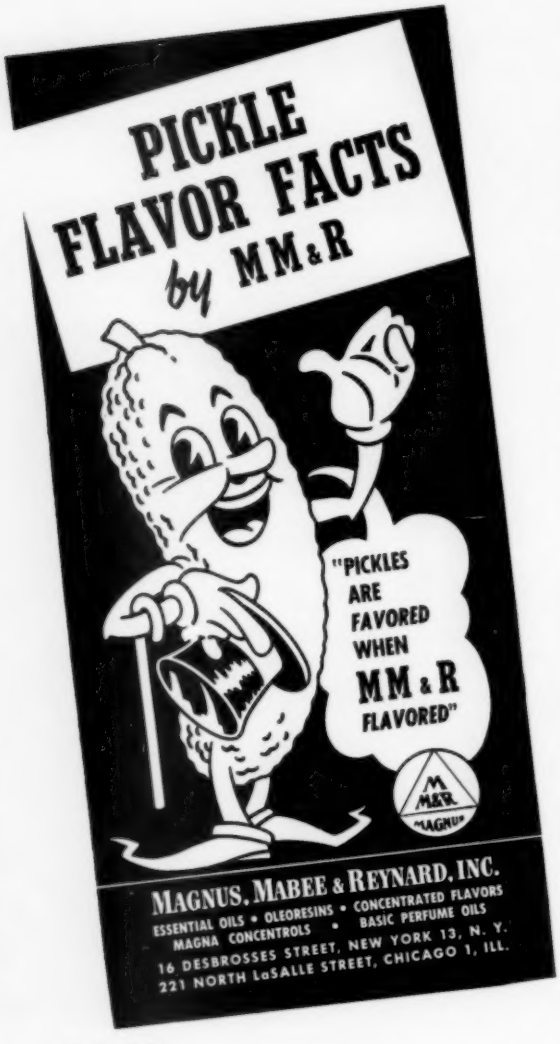
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with recomendations, there is no hazardous amount of these materials present in food products. The very few occasions where illness was attributed to a residue were the result of gross misuse of an insecticide and complete disregard of directions.

The problem of policy for a processor of adult foods may be quite as difficult as it is for a processor of baby foods, since considerable data reported shows that younger animals are more sensitive to toxic effects of insecticides than are older animals. The maximum non-toxic dosages range from 2 to 10 times less for the young animal than for the adult.

Many factors work in favor of the processor, Dr. Phillips noted. Most important among them are the extensive educational program constantly pursued by the chemical industry, the Food and Drug Administration, agricultural experiment stations, and county agents. One of the primary effects of their efforts has been to impress the grower with the necessity of following label directions in applying the correct amount of pesticide at the proper time.

To date, no single method supplies all the requirements of a control program, although there are combinations of procedures that, when used to supplement each other, can provide adequate data. These methods are: screening tests, the organic chloride, cholinesterase enzyme inhibition and bioassay methods. He also outlined approaches to the laboratory control program useful for assuring conformance of finished products to established standards. While the analysis of raw material is important, the processor should also know the effect of processing methods on the residues which may be present. For those agents which are heat stable, greater care and lower tolerances must be set.

In conclusion, Dr. Phillips asserted that despite the problems of achieving and maintaining control over residues, pesticides have contibuted greatly to our high standard of living, to the high productivity of the American farmer, and to the high level of quality of the American food supply.

W. NEW YORK

F. Bruce Krysiak of Loblaw's addressed the March meeting, held at The Park Lane, Buffalo, speaking on "Quality Standards". He discussed the decisions that management has to make with respect to raw materials, management facilities, consumer demand, distribution cost, and profits.

NORTHEAST

Seventy-seven students attended the sponsored "Students' Night" meeting in March. A total of 142 members and students enjoyed welcoming our National Treasurer and nominee for National President-Elect, Mr. Ray B. Wakefield of Gerber Products Co. A feature of the meeting was the presentation of Gerber Baby Food Scholarship award plaques to two students working in the Northeast.

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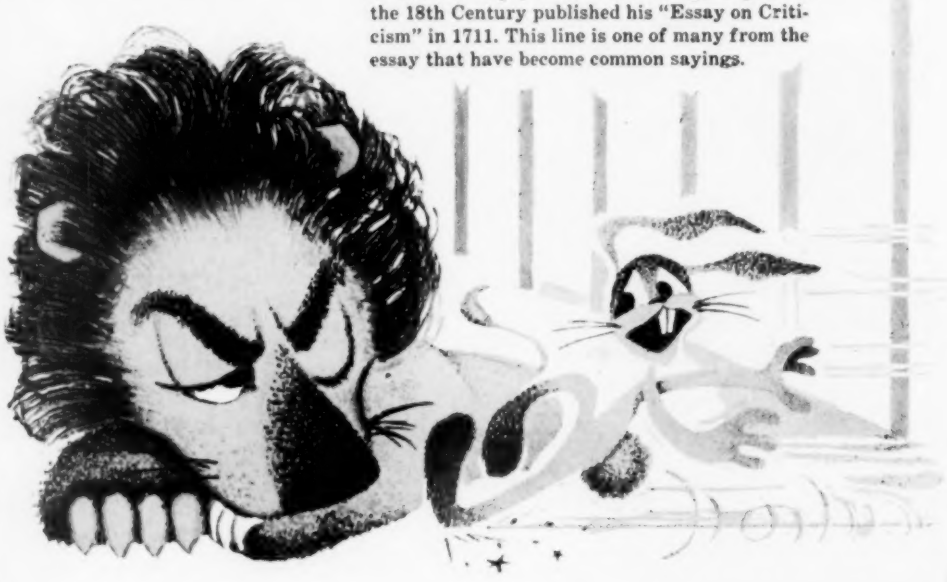

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Miss Yorkette Solomon, Department of Food Technology, University of Massachusetts, and Mr. Herbert S. Waxman, Department of Food Technology, M.I.T., received the award plaques presented by Dr. William B. Esselen and Dr. B. E. Proctor.

Mr. Wakefield spoke on "Things You Are Not Taught in School," giving sound advice to new food technologists on the importance of developing certain traits which are necessary for a successful professional life. Such things as co-operativeness, responsibility, alertness, imagination, tolerance, kindness are very important to the new technologist in finding his proper place in the food industry.

OREGON

Careers Day Report. The Oregon Section held its first "Careers in Food Technology Day" on March 29. An invitation had been sent to each Portland high school to select an outstanding student and teacher in science to be guests for the day at the Department of Food and Dairy Technology at Oregon State College in Corvallis. On arrival at the Food Technology Building in Corvallis, a special welcome was extended by Section Chairman, Oliver J. Worthington.

Twenty outstanding Portland high school students and teachers arrive by bus at the Food Technology Building, Oregon State College.

After the welcome, Harold W. Schultz, Head, Department of Food and Dairy Technology, spoke to the group on the importance of the food industry to Oregon, the United States, and the world. Dr. Schultz stressed the value of a college education, the importance of wisely choosing a major field of study, and the opportunities for bulding a rewarding career in the progressive food industry.

LIVING STANDARDS ADVANCE WITH FOOD SCIENCE AND TECHNOLOGY PROGRESS

At a special luncheon in the Student Union, Dr. Aksel Olsen, President-Elect of the IFT, presented a talk on "Progress and Food Technology". Dr. Olsen pointed out that, although food technology is relatively young among the professions, many of the great scientists such as Pasteur and Babcock could actually be classified as food technologists. He outlined how advances in standards of living have gone hand in hand with advances in food preservation technology.

In the afternoon, Clifford E. Samuels described a typical four-year college program for a food technology major. Mr. Samuels stressed that a college education only provides the scientific "tools" which then may be applied to a varied food industry. Specific job training in college is impractical, but with the scientific tools and a desire to learn, a student may fill any of hundreds of positions in the food industry. The students and teachers then toured the Food Technology Building and saw some of the current research being done on irradiated meats, freeze-dried ham, dehydrated cherries, fishery products, thermal death time studies, gas chromatography and flavor evaluation techniques.

After a milk break, during which the students were allowed to go behind the fountain counter of the dairy bar and concoct their own sundaes, the group toured the dairy processing plant. J. Orville Young and Roy W. Stein explained the processes used in the manufacture of butter, ice cream and cheese and also described some of the current research projects. They emphasized the need for more technically trained people in the dairy manufacturing industry.

The students enjoy a "do-it-yourself" ice cream sundae.

QUESTIONS AND ANSWERS

The "Career Day" ended with a very successful question and answer period. Some typical questions were: "How do starting salaries for food technologists compare with starting salaries for other professions such as chemists and engineers?" (Answer: "Definitely competitive".) "Should I major in food technology and minor in business or vice versa?" (Answer: "Aptitude tests will help in making this decision.") "What courses should I take in high school which will better prepare me for a major in food technology?" (Answer: "Don't avoid math and the sciences.") As the students and teachers left to board the bus for their return trip to Portland, they were given a packet of material extending the answers given during the day and spelling out the job opportunities. Included in the packet were the IFT pamphlet "Food Technology—Its Challenges and Rewards as a Professional Career" and the Oregon Section's pamphlet "Food Technology".

Dr. Aksel Olsen, IFT President-Elect, with the Section officers and the "Career Day" committee: from left to right, Kenneth E. Cain, Clifford E. Samuels, Jack Kay, Albert H. Bockian, Curtis J. Wilder, Oliver J. Worthington, James H. Dietz, Harold W. Schultz, Jack E. Yolland, Lois A. Sather, and Dr. Olsen.

The committee which worked so diligently to make this first Oregon "Food Technology Career Day" an outstanding success were Jack T. Yolland, Chairman, Kenneth E. Cain, Jack Kay, Albert H. Bockian, Harold W. Schultz, Clifford E. Samuels and Oliver J. Worthington. Special credit should be given Mr. Yolland for the many hours he spent working with the Portland high schools in selecting their participants.

The Oregon Section is now making plans for its Annual Meeting which will be held April 25 in Salem. This will be an all-day meeting starting at 10:00 a.m. and will feature speakers on "Food Laws and Regulations" from the Food and Drug Administration, the Oregon State Department of Agriculture and Oregon State College.

—Lois A. Sather

PITTSBURGH

The March 11 meeting of the Pittsburgh Section of the Institute of Food Technologists was attended by forty-seven members and guests. The social hour and dinner were followed by a short business meeting brought to order by Chairman Robert Buck.

After the reading and approval of the minutes of the previous meeting, Dr. C. R. Stumbo, Chairman of the Nominating Committee, announced that the following slate of nominees for 1958–59 had been drawn up and recommended to the Section.

Vice Chairman: A. N. Laubscher and Henry F. Smyth, Jr.

Secy-Treas.: Brian A. Burke, James L. Segmiller.

Councilor: I. J. Hutchings, A. W. Johnson.

Executive Committee (2): E. N. Higgins, Harry Lipman, Paul Somerville, William W. Leathen.

Following the business meeting, Dr. G. L. Reyman, Program Chairman, introduced Dr. Henry L. Cox, formerly of the Corn Products Refining Company, whose topic was "Gold from the Land of the Incas." Dr. Cox reviewed the history of corn and maize, pointing out that corn was known in the Central American region for at least 10,000 years before the arrival of such explorers as Columbus, Cortez, Balboa, and Pizzarro.

Dr. Cox also traced the stages in processing the corn from its raw state into various products such as syrup, cereals, cattle and poultry feed, starch and industrial products such as textile sizing and cooking products. The talk was illustrated with black and white and color slides.

After a brief discussion period, the meeting was adjourned.

The final meeting of this year will be held on May 13.

—John H. Rogeri

TEXAS

The Texas Section was privileged to hold two meetings during the month of March. Our first regular meeting for 1958 was held in Houston on March 6 at the Ben Milam Hotel. The formal meeting was preceded by a social dinner hour. After the business session, Mr. Ted Warner of Union Carbide Chemicals Company gave a very interesting talk on "Sorbic Acid and Its Uses in Food Preservation." A very lively discussion was carried on by informal questions and answers between the members and the speaker immediately after the formal talk.

Aksel G. Olsen

In addition to our regular meeting, and by no means a secondary meeting, the Executive Committee of Texas Section was able to meet with our members in the Dallas-Ft. Worth area for a special meeting with our President-Elect of IFT, Dr. Aksel G. Olsen. The meeting was held in Dallas on March 18 at the Dallas Hotel. The formal meeting was preceded by a social dinner hour. Following a short informal business session, Dr. Olsen spoke on "Research Planning." The talk created quite a lively discussion immediately after, which Dr. Olsen was able to handle adequately, being a former research executive with General Foods Corp.

—Jeannette White

WASHINGTON, D. C.

The third meeting of the newly formed group of Food Technologists in the Washington, D. C. area was held at the National Housing Center, 1625 L Street, on Friday, February 21, 1958. This meeting, the first meeting of the group to have a program of speakers, was preceded by two organizational meetings. Approximately eighty Food Technologists were on hand to hear a report on the Food Protection Committee; its purpose and function, organization and program, results and significance to the food industry. This subject was very ably covered by the following panel: Dr. David B. Hand, Head, Department of Food Science and Technology, Cornell University, and Vice President of the Food Protection

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Committee; Dr. Emil M. Mrak, Chairman, Department of Food Technology, University of California, and President of the Institute of Food Technologists; Dr. Frank L. Gunderson, Consultant, Washington, D. C., and representative to the Food Protection Committee's Industry Liaison Panel.

The group was then privileged to hear a report on developments in 1957-58 at the national level of the Institute of Food Technologists, by Dr. Emil M. Mrak and a report on the operation of the Institute's office by Colonel Lawrence, Executive Secretary.

This newly formed organization has now met the qualifications for application to becoming a regional section in the Institute of Food Technologists, having held three meetings, elected officers, and adopted By-Laws. At this meeting, over sixty Food Technologists became dues-paying members of the new section, and this number is being increased through mail applications for membership. The section is prepared to apply for approval as a regional section and hopes for approval before the next annual meeting of the Institute.

Officers and Committee Chairman of the new section are: Dr. Charles H. Mahoney, Chairman, National Canners Association; Mr. William J. Hoover, Vice Chairman, Chairman, Program Committee, Corn Industries Research Foundation; Mrs. Elsie H. Dawson, Secretary, Human Nutrition Research, USDA; Dr. Robert C. Wiley, Treasurer, University of Maryland; Dr. Frank L. Gunderson, Councilor; Dr. F. Weiss, Executive Committee, Scientific Consultant; Dr. John R. Matchett, Executive Committee, U. S. Department of Agriculture; Mr. H. P. Schmitt, Executive Committee, National Association of Frozen Food Packers; Captain R. I. Pomerantz, Executive Committee, Office of Quartermaster General; Mr. John I. Thompson, Chairman, House Committee, Consultant; Mr. Allen A. Kraft, Chairman, Publicity Committee, USDA, National Research Center, Beltsville, Maryland.

Seated, l. to r.—Colonel Charles Lawrence, Executive Secretary, IFT; Dr. Emil Mrak, President, IFT, University of California; Mrs. Elsie H. Dawson, Section Secretary, Human Nutrition Research, USDA; Mr. Frank L. Gunderson, Councilor, Consultant; Dr. David Hand, Department of Food Technology, Cornell University; Dr. Charles H. Mahoney, Chairman of the new section, National Canners Association. Standing, l. to r.—Mr. William J. Hoover, Vice Chairman of the Section, Corn Industries Research Foundation; Dr. Robert C. Wiley, Treasurer, University of Maryland.

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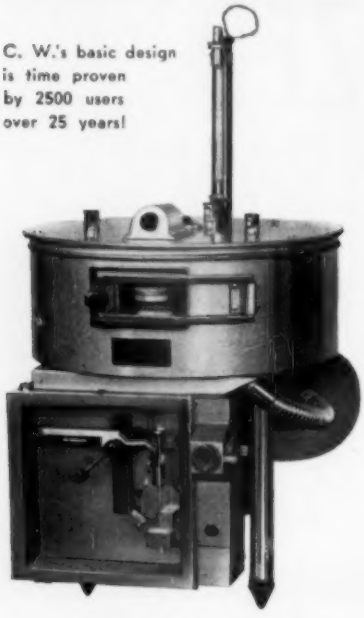

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Literature

BOOKS

CEREAL LABORATORY METHODS. 6th Edition, 1957. Edited by Emery C. Swanson. American Association of Cereal Chemists, Inc.

The objectives set forth in the preface of this new edition of Cereal Laboratory Methods have been excellently achieved. A completely new style of presentation has been followed. The book is divided into 100 categories consisting of "determinations" which are arranged in alphabetical order, starting with the determination of acid and ending with yeast strength.

The broad categories are divided into individual methods; for example, 31.0 Fat, 31.1 Fat, in Cocoa, 31.2 Fat, in dog food (baked), 31.6 Fat, in wheat flour, etc. Many new methods have been included which were not available previously. Among these are methods for testing of prepared mix ingredients, physical properties of doughs, etc. Information for the preparation and standardization of solutions is combined under one category.

This edition is highly recommended as a laboratory manual in all food industries that deal with the analyses of cereal items. The new style of presentation makes it especially easy to use by both the chemist and the technician.

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INDUSTRY PUBLICATIONS

Bulletin No. 959, recently issued by the Pfaudler Co., a division of Pfaudler Permutit Inc., Rochester, N.Y., describes the firm's new 21-station rotary piston filler Model RPC-21. The unit fills 500 containers a minute, with such features as "no container, no fill" mechanism, simplified changeover for different container sizes, and fill adjustment over full range of container capacity without replacing cam track. The bulletin may be obtained by writing the firm at 1059 West Ave. in Rochester.

A newly revised 32-page reference guide has been released to the meat industry by Merck & Co., Inc., Rahway, N.J. The booklet gives detailed information on the action, use and benefits of Neo-Cebicure and Neo-Cebitate in the treatment of cured meats and in the spraying of sliced, cured meat products. Particular emphasis is given to the color advantages afforded by Sodium Isoascorbate and Isoascorbic acid, and the economic advantages for meat packers using these products. The brochure, which is available from the company on request, also contains reproductions of federal memoranda governing both the use of the products and labeling requirements for treated meats.

A new article on the multi-billion dollar research problem of fat rancidity has been published by Evans Research and Development Corp. "A Nice Fat Problem" is the latest in the "Research Comments" series. Included are current theories on rancidity, the present status of research on anti-oxidants, and the expected progress in combatting this problem.

Technical bulletin 90-110-13, "Control Consistency with a Flow Bridge," discusses theory and operation of flow bridge for measuring consistency of slurries such as tomato paste. Free copies may be obtained by writing Fischer & Poter Co., 544 Jacksonville Rd., Hatboro, Pa.

A new eight-page catalog, just published by Dodge & Olcott, Inc., 180 Varick St., New York 14, N.Y. lists a group of flavoring and perfume compounds approved by the Orthodox Union of Jewish Con-

gregations of New York, Inc. Products listed include essential oils, balsams, oleoresins, aromatic chemicals, terpeneless oils, resinexes, flavor and seasoning bases, and perfume compounds. Copies are available upon request.

Proceedings of Symposia on Military Subsistence Problems Available in Limited Quantity

The following *Proceedings* of symposia on problems of importance in food research and development are available in limited quantity to research organizations in the food fields:

- Contributions of Browning Research to Ration Item Stability
- Dry Whole Milk
- Precooked Frozen Foods
- Yeast, Its Characteristics, Growth and Function in Baked Products
- Chemistry of Natural Food Flavors
- Methods for Evaluation of Nutritional Adequacy and Status
- Establishing Optimum Conditions for Storage and Handling of Semiperishable Subsistence Items
- Vacuum Requirements for Canned Tomatoes
- Low Temperature Test Methods and Standards for Containers
- Monosodium Glutamate—a symposium (March 1948)
- Monosodium Glutamate—a second symposium (September 1955)

Food scientists at work in one or more of these fields are invited to look over the list and order in accordance with their needs. The symposia were sponsored in most instances by the National Research Council's Committee on Food.

The *Proceedings* of each symposium are published in full and consist of formal papers, delivered by leaders in the field represented, and the essential discussion.

Application for copies should be made to the Commandant, Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, 1819 West Pershing Road, Chicago 9, Illinois.

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LITERATURE OF FOOD RESEARCH AND TECHNOLOGY [1]

Commodities

Aspects techniques de l'expertise des conserves. Henri Cheftel. Paris, Gauthier-Villars, 1957. 55 p. Établissements J.-J. Carnaud et Forges de Basse-Indre. Laboratoire de recherches. Bulletin no. 13.)

Composition and therapeutic properties of honey. U. S. Department of Agriculture Library. Beltsville, Md., 1956. 11 p.

Insect control in flour mills. R. T. Cotton. Washington, U. S. Govt. Print. Off., 1958. 30 p. (U. S. Department of Agricultural handbook no. 133.) pap. $0.15.

Home-made cheeses, a "Farm woman's library" book. New York, Sport Shelf, 1957. 55 p. pap. $1.25. Hints for a competitor.

Maple sirup producers manual. C. O. Willits. Washington, U. S. Govt. Print. Off., 1958. 80 p. (U. S. Department of Agriculture. Agricultural handbook no. 134.) From the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. D. A.

Yeasts. W. Roman, ed. New York, Academic Press, 1958. 246 p. $7.50. Description of the chemical makeup of yeasts used for various purposes, such as baker's yeast, brewer's yeast, wine and fruit yeasts, sake yeasts, food yeasts and yeast preparations. An import. (To be reviewed in detail in this journal.)

Zucker und Zuckerwaren. Albrecht Fincke. Berlin, Hayn, 1957. 132 p. Grundlagen u. Fortscheitte d. Lebensmitteluntersuchtung. Bd. 5.) 19.50 marks.

Food Engineering

Amerikanische Kältetechnik; Bericht über d. Ergebnisse e. Vortrags-u. Studienreise 1955/56. Rudolph Plank. Karlsruhe. C. F. Müller, 1957. 20 p. pap. 4.00 marks. A report of a trip by a versatile German student of refrigeration.

Effect of dehydration, preparation, and storage on vitamin content of beefsteaks. Chicago, Quartermaster Food and Container Institute for the Armed Forces, 1957. 9 p. processed. Available from the Office of Technical Services, U. S. Department of Commerce, Washington 25. Refer to PB 131294. pap. $0.50.

[1] Material for this list should be sent to Dr. James G. Hodgson, Chief, Library Branch, Quartermaster Food and Container Institute for the Armed Forces, 1819 W. Pershing Road, Chicago 9, Illinois.

Process engineering in the food industries. R. J. Clarke. New York, Philosophical Library, 1958. 363 p. $10.00. An imported publication which deals with plants and equipment in use in Great Britain.

Research on the refrigeration of perishable commodities; Annual report of the Director, 1957. Colorado Springs, Colo., Refrigeration Research Foundation, 1958 (?). 44 p. pap. apply. Includes a list of publications, and Grants-in-aid for 1957. The Foundation is at 12 North Meade Ave., Colorado Springs.

Snap beans for marketing, canning and freezing. W. J. Zaumeyer. Washington, U. S. Govt. Print. Off., 1957. 16 p. (U. S. Department of Agriculture. Farmer's bulletin, 1915.) Revised Sept. 1954, slightly revised Sept. 1957.

Chemical Aspects

Chemistry of lipids of biochemical significance, 2nd ed. rev. J. A. Lovern. London, Methuen, 1957. 172 p. Methuen's monographs on biochemical subjects.) 12 s, 6 d. Deals with structure of lipids, their preparation and analysis in tissue, with "turnover" and with the function of lipids.

Chemistry of the steroids. Charles W. Shoppee. New York, Academic Press, 1958. 321 p. (Organic chemistry monographs.) $9.00.

Elemente der fünften Hauptgruppe. Stickstoff. von Wolfgang Leithe. Berlin, Springer, 1957. 244 p. (Wilhelm Fresenius and Gerhart Jander, eds. Handbuch der analytischen Chemie. Tom. 3, Qualatative bestimmungs-u. Trennungsmethoden. Bd. 5a.) Unbd. 54 Marks.

Macromolecules in cell structure. Albert Frey-Wyssling. Cambridge, Mass., Harvard University Press, 1958. 119 p. (John M. Prather lectures, 1956.) $5.00. The fine structures of starch, plant-cell walls, chloroplasts, and other related material.

Molecular structure and organoleptic quality; comprising papers read at a Symposium organized by the Overseas Section held in Geneva 2–3 May 1957. New York, Macmillan, 1958. 127 p. (Society of Chemical Industry. Monograph no. 1.) $3.75. Includes papers on the relationship between molecular structure and odor.

Naturally occurring quinones. R. H. Thompson. New York, Academic Press, 1958. 309 p. (Organic chemistry monographs.) A query into the chemistry of quinones proper and related quinols and anthrunes, the largest class of natural coloring matter. An import.

New research on animal fats and proteins. O. H. M. Wilder. Chicago, 1958. 13 p. (American Meat Institute Foundation. Circular no. 42.) pap. apply.

Biological and Physical Aspects

Biological ultrastructure. Arne Engstrom and J. B. Fineau. New York, Academic Press, 1957. 335 p. $8.00. Describes methods, principles of molecular structure, and covers the role of proteins, lipids, carbohydrates, nucleic acids, and mineral salts.

Chronic radiation hazards. G. J. Neary and others. New York, Pergamon Press, 1957. 191 p. $7.50.

A bibliography of bacterial spores. ed by H. A. Frank and L. L. Campbell, Jr., Washington Agr. Exp. Stations, Inst. Agr. Sci., Station Circ. 323 (Feb. 58) Pullman, Wash. "Qualified persons may obtain copies as long as they last."

Radiation laboratory techniques. R. A. Faires and B. H. Parks. New York, Pitman, 1958. 256 p. $5.75. For those handling isotopes in the laboratory. An import.

Safety techniques for radioactive tracers. J. C. Boursnell. New York, Cambridge University Press, 1958. 78 p. $1.75.

Miscellaneous

Applied differential equations. Murray R. Spiegel. Englewood Cliffs, N. J., Prentice-Hall, 1958. 396 p. (Prentice-Hall math. series.) Background for statistical work.

British pharmacopoeia, 1958. London, Pharmaceutical Press, 1958. 1012 p. 63 s. Issued by the General Medical Council. Comparable to the U.S.P. but useful when the English use a different terminology.

Microdiffusion analysis and volumetric error. 4th rev. ed. Edward J. Conway. New York, Macmillan, 1958. 483 p. $8.50. Largely concerned with the analysis of ammonia and urea, but is listed as expounding a new treatment of volumetric error, with particular reference to microquantities. An import.

Scientific societies in the United States, 2nd ed. Ralph S. Bates. New York, Columbia University Press, 1958. 306 p. $6.50. A Technology Press, Mass. Inst. of Technology publication. First issued in 1945, this edition has a new chapter covering changes in scientific societies since the end of World War II.

The wonderful world of food, the substance of life. John Boyd Orr, 1st baron Boyd-Orr. Garden City, N. Y., Doubleday, 1959. $3.45. A history of the quest for food. Author was first Director-General of the Food and Agriculture Organization of UN.

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ABSTRACTS

—Compiled by H. A. Campbell

ANALYTICAL METHODS

A paper chromatographic procedure for the separation and identification of coumarin, dihydrocoumarin (melilotin), and methylcoumarin.

MITCHELL, L. C. (Dept. of Health, Education, and Welfare, Washington, D. C.). *J. Assoc. Offic. Agr. Chem. 40*, 1029–33 (1957).

A paper chromatographic procedure is described for the sepn. and identification of coumarin, dihydrocoumarin and methylcoumarin after they have been isolated from food materials in essentially pure condition.

A quantitative paper-chromatographic study of the amino acid composition of proteins and juice of the orange.

WEDDING, R. T. AND SINCLAIR, W. B. *Bot. Gaz. 116*, 183–8 (1954).

A quant. paper-chromatographic analysis of the amino acid compn. of acid hydrolyzates of proteins sepd. from Washington Navel and Valencia oranges, and of the centrifuged juice of these fruits, indicated that both qual. and quant. differences exist between the proteins from the 2 sources. The most striking differences were the absence of ornithine in the Valencia orange protein and of γ-aminobutyric acid in the Navel orange protein. Not all the amino acids found in the hydrolyzates could be detected in the free form in the juice. There were also marked differences in amino acid and amide compn. of the 2 juices, but they did not reflect differences in the compn. of the 2 proteins.

The use of selective desorption from carbon columns for the determination of dextrose and maltose in starch conversion products.

PATTERSON, S. J. AND SAVAGE, R. I. *Analyst 82*, 812-18 (1957).

A method is proposed for detg. the mono- and disaccharides in starch conversion products, based on selective desorption followed by elution from a carbon column. Recovery is quant., provided the most suitable temp. conditions for recovery from each batch of carbon are established, before it is put into routine use. The method of testing the carbon in order to obtain these conditions is described.

Determination of food tannins by ultraviolet spectrophotometry.

OWADES, J. L., RUBIN, G. AND BRENNER, M. W. (Schwarz Labs., Inc., Mount Vernon, N. Y.). *J. Agr. Food Chem. 6*, 44–8 (1958).

A direct procedure for the detn. of tannins in beer and tea by measuring the u.v. absorption is described. The tannins are extd. with ethyl acetate, and after evaporation of solvent are taken up in acidified methanol and read on Beckman D.U. The method should be standardized for the specific tannins in each food.

BIOLOGICAL SCIENCES

Biochemistry

Studies on the availability of amino acids.

GUPTA, J. D. (Univ. of Wisconsin, Madison). *Dissertation Abstr. 17*, 2405 (1957).

The availability of tryptophan, lysine and threonine from different proteins was estd. by comparing the growth response obtained with the protein as a test material supplementing a diet deficient only in that amino acid with the responses obtained when the deficient diet was supplemented with graded levels of the cryst. amino acid. A study was made also of the rate of protein digestion *in vivo*. The purified proteins studied were: casein, defatted beef and pork, egg albumin, gelatin, fibrin, a soybean protein, zein and wheat gluten. Three cereals were tested: rice, wheat flour and corn.

Precursors of squalene and cholesterol.

DITURI, F., RABINOWITZ, J .L., HULLIN, R. P. AND GURIN, S. (Dept. of Biochem., School of Med. Univ. of Pennsylvania, Philadelphia). *J. Biol. Chem. 229*, 825–36 (1957).

Homogenates of rat liver have been shown to incorporate 2-C^{14}-mevalonic acid into squalene and cholesterol. Biosynthetic C^{14}-squalene was ozonized and the radioactivity of the cleavage products detd. The results suggest that mevalonic acid is incorporated directly into squalene with little randomization of isotope before polymerization. The evidence also suggests that condensation occurs between carbon atom 5 of 1 molecule and carbon 2 of a 2nd molecule, with decarboxylation possibly taking place during or after polymerization.

Some physical and chemical properties of the ribonucleic acid contaminant of rabbit muscle myosin preparations.

MIHALYI, E., BRADLEY, D. F. AND KNOLLER, I. (Natl. Heart Inst., Bethesda, Md.). *J. Am. Chem. Soc. 79*, 6387–93 (1957).

Myosin B isolated from rabbit muscle by standard methods contains up to 1% ribonucleic acid (RNA) by wt. as an impurity. This paper describes procedures for sepg. this RNA from the protein as well as some of its phys. and chem. properties.

Dependence of protein synthesis on ribonucleic acid synthesis. II. Nonparticipation of preformed ribonucleic acid in protein synthesis.

BEN-ISHAI, R. (Weizmann Inst. of Sci., Rehovoth, Israel). *Biochim. Biophys. Acta 26*, 477–83 (1957).

Systems were investigated in which RNA could be synthesized in the absence of protein formation and in which protein synthesis could subsequently be induced. It has been shown that the preformed RNA thus obtained participates neither in general protein synthesis nor in induced enzyme (β-galactosidase) formation. The results obtained in the present study lead to the conclusion that only the RNA formed simultaneously with protein participates in the synthesis of the protein.

Interrelations between nucleic acid and protein biosynthesis. I. Synthesis and fate of bacterial nucleic acids during exposure to, and recovery from the action of chloramphenicol.

HAHN, F. E., SCHAECTER, M., CEGLOWSKI, W. S., HOPPS, H. E. AND CIAK, J. (Walter Reed Army Medical Center, Washington, D. C.). *Biochim. Biophys. Acta 26*, 469–76 (1957).

Cells of *E. coli* B/r, whose protein synthesis is inhibited by chloramphenicol, accumulate several times the amts. of intracellular nucleic acids contd. in log-phase bacteria before the onset of chloramphenicol-induced bacteriostasis. Upon removal of chloramphenicol the bacteria pass through a recovery phase during which much of the excess of nucleic acids is ejected from the cells before growth and multiplication are resumed. It is thought that these conditions results from the imbalanced synthesis of normal nucleic acids under the influence of chloramphenicol rather than from the formation and elimination of abnormal polynucleotides.

Novobiocin. VI. Structure of the coumarin moiety.

STAMMER, C. H., WALTON, E., WILSON, A. N., WALKER, R. W., TRENNER, N. R., HOLLY, F. W. AND FOLKER, K. (Merck Sharp & Dohme Research Labs., Div. of Merck & Co., Inc.). *J. Am. Chem. Soc. 80*, 137–40 (1958).

The structure of the coumarin moiety of novobiocin is shown to be 3-amino-4, 7-dihydroxy-8-methylcoumarin. Thus, the structure of novobiocic acid, the aglycon of novobiocin, is 2,7-dihydroxy-3(4-hydroxy-3-[3-methyl-2-butenyl]-benzamido)-8-methylchromone.

Microbiology

Antimicrobial factors of normal tissues and fluids.

SKARNES, R. C. AND WATSON, D. W. (Dept. of Bacteriol. and Immunol., Univ. of Minnesota, Minneapolis). *Bacteriol. Rev. 21*, 273–87 (1957).

The role of antimicrobial tissue factors in resistance may represent one facet of the whole defense system. We do not suppose that these substances exist, as such, in normal tissues but that they arise in response to physiol. changes which accompany stress. They may act in the body to kill or to slow the growth of invading microorganisms, enabling other host defenses to operate more efficiently in the removal of inimical agents from the tissues. Allied mechanisms of natural resistance to infectious disease, with

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which the antimicrobial factors may be integrated, have been well reviewed.

Symposium on preparation of samples for microbiological assay.

SKEGGS, H. R. (Merck Inst. for Therapeutic Research, West Point, Penna.). *Bacteriol. Rev. 21*, 257–8 (1957).

Many of the problems encountered in obtaining satisfactory analyses are due, not to the assay procedure *per se*, but to difficulties in prepn. of the sample for assay. This symposium was organized to focus attention on some of the problems encountered, and to discuss a few of the specific methods which have been extensively studied.

Protoplasmic differences between mesophiles and thermophiles.

KOFFLER, H. (Dept. of Biol. Sci., Purdue Univ., West Lafayette). *Bacteriol. Rev. 21*, 227–32 (1957).

It is shown that the cytoplasmic proteins of thermophiles are markedly more stable than comparable prepns. from mesophiles. Using flagella (which are polymers of fibrous proteins) as models, we have found clues that might explain the remarkable heat stability of thermophile proteins. These studies though still rudimentary, illustrate the mol. basis of biol. differences.

Symposium on problems in the microbiological analyses of foods.

APPLEMAN, M. D. (Dept. Bacteriol., Univ. of Southern California, Los Angeles). *Bacteriol. Rev. 21*, 241–4 (1957).

Dr. Dack discussed the philosophy of the microbiol. analysis of foods, which in part demands that enforcement agencies educate processor groups for the greatest over-all gain in food processing. Certain pathogens, e.g. *Salmonella*, are naturally present in some foods, such as frozen, diced eggs and meat. Dr. Gunderson discussing sampling problems in foods showed that there was no direct relationship between microbiol. counts in a good product as finished at the processing plant and that purchased by the consumer. Dr. Silliker discussed the selection of bacteriol. methods showing that bacteriol. methods are less precise than anal. chem. methods for most foods and that the significance of the bacteriol. methods is not as readily interpreted as the chem. Dr. Hobbs discussed the sources and multiplication of pathogens in foods as an integral part of the microbiol. analysis of foods emphasizing that public health measures have reduced the incidence of water, milk and food-borne disease. Dr. Mallman discussed indicator species that have been used to indicate pollution in water supplies and how the problem differs to some extent in the microbiol. analysis of foods. Dr. Thatcher in discussing the topic "What May Microbiological Standards Mean" showed the complexity and the subjectiveness involved. Dr. Moss discussed the microbiol. safety of foods stressing the importance of recognizing that foods substandard from bacteriol. aspects may be more important as a risk to consumer health than food substandard from chem. compn.

Nutrition

Physical activity, serum cholesterol and other lipids in man.

TAYLOR, H. L., ANDERSON, J. T. AND KEYS, A. (Lab. of Physiol. Hygiene, School of Public Health, Univ. of Minnesota, Minneapolis). *Proc. Soc. Exptl. Biol. Med. 95*, 383–6 (1957).

The effect of increasing the level of daily phys. activity on the serum cholesterol concn. was studied in 9 clinically healthy univ. students. The phys. activity was increased by walking on the motor-driven treadmill at a rate which required 1280 cal. for 2 hrs. The caloric intake was increased by 900 cal. but the proportion of calories derived from fat was held const. There was no change in the serum chlosterol concn. It is concluded that the results are consistent with the hypothesis that the serum chlosterol concn. is related to the proportion of total calories derived from fat.

Role of dietary fat in human nutrition. III. Diet and the epidemiology of coronary heart disease.

KEYS, A. AND GRANDE, F. (Lab. of Physiol. Hygiene, School of Public Health, Univ. of Minnesota, Minneapolis). *Am. J. Public Health 47*, 1520–30 (1957).

The authors present data supporting an assocn. between the incidence of coronary artery disease, a high intake of total dietary fat and the elevation of serum cholesterol. The data indicate that the more common fats of the American diet, when eaten in large amts., may contribute to production of relative cholesterolemia and so to atherogenesis. There is suggestive evidence that fatty meals may induce hypercoagulability of the blood and inhibition of fibrinolysis.

Role of dietary fat in human nutrition. IV. Experimental and clinical evidence relating to the effect of dietary fat upon health in man.

VAN ITALLIE, T. B. (Dept. of Nutrition, Harvard Univ. School of Public Health, Boston, Mass.). *Am. J. Public Health 47*, 1531–6 (1957).

Current exptl. and clin. studies suggest that hypercholesteremia and, presumably, atherosclerosis tend to occur when the dietary intake of fat is high, 40% of total calories or more, or when persons remain in pos. caloric balance. The evidence also indicates that the hypercholesteremic response to satd. fats may be mitigated or even nullified if adequate amts. of highly unsatd. fats are concurrently present in the diet. The hypothesis that the hypercholesteremia of adult males in northern Europe and North America is due to a chronic relative deficiency of essential fatty acids has certain attractive features; yet, the exptl. evidence currently available only partly supports such a concept.

Vitamin A deficiency in the rat, with and without added dietary cholesterol.

GREEN, B., LOWE, J. S. AND MORTON, R. A. (Dept. of Biochem., Univ. of Liverpool, Engl.). *Biochem. J. 67*, 223–8 (1957).

Weanling rats on a vitamin A-deficient diet were compared with litter mates on the same diet to which cholesterol (2%) had been added. The examn. of tissue unsaponifiable fractions showed little evidence of interaction between cholesterol feeding and vitamin A deprivation.

Intakes of vitamins A and D.

Nutrition Revs. 15, 74–5 (1957).

A review of the literature on the intake of vitamins A and D. It is noted that the rise in the incidence of idiopathic hypercalcemia in infants appears to be due to high intake of vitamin D and Ca. The need of a critical study of the effect of moderately high intakes of the essential nutrients is stressed.

Human utilization of dehydroascorbic acid.

SABRY, J. H. (Pennsylvania State Univ., State College). *Dissertation Abstr. 17*, 2972 (1957).

The utilization of dehydroascorbic acid by humans was measured by a comparison with that of reduced ascorbic acid. By the criterion of plasma ascorbic acid, the 2 intake forms of the vitamin were utilized to the same extent. On the basis of total ascorbic acid retention, the highest relative utilization of dehydroascorbic acid occurred when the intake of this form was lowest. However, comparable blood levels of the vitamin on both forms of intake were maintained.

Effect of calcium and phosphorus salts on the utilization of iron by anemic rats.

CHAPMAN, D. G. AND CAMPBELL, J. A. (Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Canada). *Brit. J. Nutrition 11*, 127–33 (1957).

Diets made up of 80% bread, to which was added bone meal, $CaCO_3$, calcium lactate, $CaCl_2$, disodium phosphate or comsodium hexametaphosphate have been fed to groups of 10 anemic rats for 10-week periods. Each of the Ca salts was found to interfere with the utilization of Fe as reflected by liver Fe stores, hemoglobin regeneration and increase in heart wt. The two phosphates had no significant effect on either the Fe content of the liver or on the rate at which hemoglobin was regenerated. The interference caused by the Ca salts is discussed on the basis that the "mucosal block" may be operating to prevent the absorption of Fe.

Quantitative nutritional studies with water-soluble, chemically defined diets. III. Individual amino acids as sources of "non-essential" nitrogen.

BIRNBAUM, S. M., WINITZ, M. AND GREENSTEIN, J. P. (Natl. Cancer Inst.,

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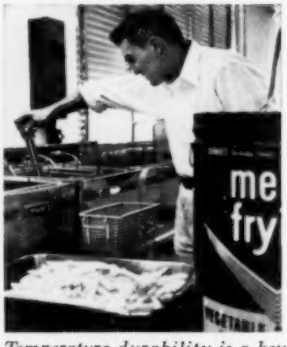

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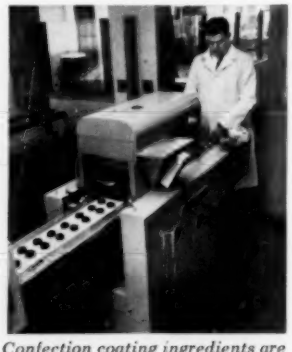

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Bethesda, Md.). *Arch. Biochem. Biophys. 72*, 428–36 (1957).

Various amino acids and other nitrogenous compds. were individually added to the basal diet at the expense of an equal amt. of the glucose component, and the resulting diets, offered *ad libitum* to weanling rats. These supplemented diets were all isonitrogenous, i.e., either 25.3 or 22.0 g. total N/kg. The diets providing the best growth, about 3 g./day, were those in which L-alanine ammonium L-glutamate, L-glutamine, ammonium L-aspartate, and L-proline were individually furnished as the sole source of "nonessential" N. Ammonium acetate proved more effective in promoting growth than either urea or glycine, while L-serine, L-hydroxyproline, and L-cysteine, at the levels used (15.7 g. total N/kg. diet) proved to be toxic to the animals.

The development of a semi-synthetic diet and its use in a study of protein metabolism in the cat.

Miller, S. A. (Rutgers Univ., New Brunswick, N. J.). *Dissertation Abstr. 17*, 2142 (1957).

Studies, on a diet characterized by a relatively high protein, caloric density and vitamin content, show that the nitrogen/calorie ratio is higher in the cat than in the dog, which can mean that the cat has a more rapid rate of protein metabolism. The indexes for maintenance in the adult cat for egg albumin, casein and wheat gluten were 1, 0.9, and 0.7, resp. Casein diets supplemented with methionine indicated that, while casein had sufficient methionine for maintenance in the adult cat, the kitten required a greater amt. of methionine for growth. No change was observed in creatinine excretion even with apparently excessive levels of methionine supplementation. The lack of toxic symptoms upon feeding excess sulfur amino acids could be due to the presence of a detoxification mechanism peculiar to the cat, converting methionine to cysteine, which is subsequently conjugated with isoamyl alc. to form felinine.

The role of protein in diets for weight reduction.

Dole, V. P. (Hosp. of the Rockefeller Inst., New York, N. Y.). *Am. J. Clin. Nutrition 5*, 591–6 (1957).

Some evidence indicates that obese patients can safely lose N during wt. reduction. It suggests that some loss of N may be a proper part of reduction, since the obese subject probably has more than a normal amt. of N in his body. Although in principle it is clear that the aim of a reduction diet should be a normal body compn., as well as a normal wt., there are no reported studies that relate the proportions of different reduction diets to changes in body compn. Thus, a high-protein diet has no proven advantage over simple quant. limitation of a diet contg. a normal proportion of protein.

The specific dynamic action of food and the satiety mechanism.

Passmore, R. and Ritchie, F. J. (Dept. of Physiol., Univ. of Edinburgh, Scotland). *Brit. J. Nutrition 11*, 79–85 (1957).

Energy exchange in man during the 1st hr. after a meal has been investigated. A rise in rectal temp. and a probable rise in av. skin temp. have been demonstrated, but these were small. Oxygen consumption also increased immediately after a meal. The results are consistent with Brobeck's (1948) hypothesis that increased heat production is an important part of the satiety mechanism.

Nutrition and cancer.

Nutrition Revs. 15, 178–9 (1957).

A review of how diet, in animals and man, affects the incidence of spontaneously occurring and induced cancer. It is recommended that the avoidance of overweight is probably one way to prevent, or delay, the appearance of some malignant processes.

Physiology

Studies in the intravenous injection of tocopherol in chickens and of interactions of tocopherol with proteins and amino acids.

Voth, O. L. (Pennsylvania State Univ., State College). *Dissertation Abstr. 17*, 2799 (1957).

A study of the effect of intravenously injected tocopherol suggests that tocopherol is rapidly removed from the blood stream by the liver from which it may then be slowly released to the circulation for transport and deposition in other tissues. Overall electrostatic charge appears to be the most important single factor in the binding of tocopherol by proteins.

Absorption of various fluorine compounds from the gastrointestinal tract of the rat.

Zipkin, I. and Likins, R. C. (Natl. Inst. Dental Research, Natl. Inst. Health, Bethesda, Md.). *Am. J. Physiol. 191*, 549–50 (1957).

Recent studies have indicated that NaF, Na_2SiF_6, Na_2PO_3F and SnF_2 are similar in their ability to inhibit caries and to be deposited in the bones and teeth. The absorption of these variously bonded F compds. from the gastrointestinal tract of the rat was detd. NaF, Na_2SiF_6, Na_2PO_3F and SnF_2 were absorbed to the same extent (48%), whereas KPF_6, $EtNPF_6$ and KBF_4 were absorbed to a significantly greater degree (74%). The results suggest that covalently held F compds. are absorbed at a greater rate than the F ion.

The subcommissural organ: a regulator of thirst.

Gilbert, G. J. (Johns Hopkins Hosp., Baltimore, Md.). *Am. J. Physiol. 191*, 243–7 (1957).

Electrocoagulative destruction of the subcommissural gland area of the midbrain in rats results within a few days in death due to dehydration. Subcutaneous injection of aq. exts. of rat or beef subcommissural area into rats resulted in sharp depression of H_2O consumption, with a rise in intake on the 2nd day. It is suggested that the active substance may be a large, readily denatured protein.

Interpretation of certain changes in body water and electrolytes.

Darrow, D. C. and Hellerstein, S. (Dept. of Pediatrics, Univ. of Kansas Med. School, Kansas City). *Physiol. Revs. 38*, 114–35 (1958).

This review presents body compn. data and discusses certain factors determining body compn., especially with respect to H_2O, Na, K and Cl. Determination of body compn. *in vivo* involves many errors. Ideally H_2O and electrolytes should be expressed in terms of unit of fat-free, bone salt-free solids. Even with this reference one must consider the possible variations in the proportions of extracellular and intracellular solids.

Age changes in some physiologic processes.

Shock, N. W. (Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.). *Geriatrics 12*, 40–8 (1957).

Objective evidence is presented to illustrate the following points about aging: (1) not all physiologic functions change with age, (2) wide individual differences exist in the effects of age, (3) some of the age changes in physiologic functions of the total person can be ascribed to a loss of cellular protoplasm, (4) there is some evidence that cellular functions change with age, and (5) our ultimate goal is to identify the cellular properties that are the basis to the biologic process of aging which is common to all living animals.

Body and organ weights of rats during acclimatization to an altitude of 12,470 feet.

Timiras, P. S., Krum, A. A. and Pace, N. (Dept. Physiol., Univ. of Calif., Berkeley). *Am. J. Physiol. 191*, 598–604 (1957).

Body and organ wts. of sea level control rats were compared with those of rats (P) born at sea level and then exposed to 12,470 feet, and with those of rats (F_2) of the second filial generation born and maintained at altitude. Body wt. was significantly lower from the fifth day of age in the F_2 rats than in either control or P animals. Elevated hemoglobin and hematocrit values were accompanied by marked cardiac hypertrophy in all F_2 rats. In the P animals hemoglobin and hematocrit values were elevated after 2 months of exposure and reached a plateau after 6 months. Adrenal wt. was markedly increased in all P animals but not in the F_2 rats. In the P rats at altitude for 24 and 72 hrs. adrenal enlargement was accompanied by thymic and lymphatic atrophy.

FOOD AND FOOD TECHNOLOGY

Baking and Bakery Products

White rings in frozen bread.

PENCE, J. W., STANDRIDGE, N. N., BLACK, D. R. AND JONES, F. T. (Western Utilization Research and Devel. Div., U. S. Dept. of Agr., Albany, Calif.). *Cereal Chem. 35*, 15–26 (1958).

During storage of bread in the frozen state, a whitened or opaque ring ⅛ to ¼ in. wide developed in the crumb just beneath the crust. Length of storage before initial occurrence and subsequent rate of inward growth of the ring were dependent on the storage temp. The whitening phenomenon is assocd. with a movement of moisture by sublimation. The effect can be produced by lyophilization of frozen crumb, provided its moisture content is above about 37%.

Cereals and Grains

Amino-acid composition of commercial samples of a high-protein and a low-protein wheat flour.

BLOCK, R. J. AND MANDL, R. H. *Contribs. Boyce Thompson Inst. 18*, 477–82 (1957); *Chem. Abstr. 52*, 1498 g (1958).

The amino acids obtained by hydrolysis of com. samples of wheat flour were compared by means of quant. paper chromatography. The anal. results indicate that there are differences in the amts. of cystine, valine, glutamic acid, and proline in the total proteins of the wheat flours. No significant differences were found in the remaining amino acids present, including lysine.

A revised thiochrome procedure for the determination of thiamine in cereal products.

BECHTEL, W. G. AND HOLLENBECK, C. M. (Amer. Inst. of Baking, Chicago, Ill.). *Cereal Chem. 35*, 1–14 (1958).

A procedure for the detn. of thiamine in all cereal products is presented. This improves and simplifies the procedures given in the sixth edition of Cereal Laboratory Methods. Results from 3 series of collaborative tests of a wide variety of cereal products show that the new procedure gives results at least equal in precision to those of other methods.

The nutritive value of rice and its by-products.

KIK, M. C. (Agr. Expt. Sta., Univ. of Arkansas, Fayetteville). *Rice J. 60*, 28 (Nov. 1957).

A summarizing article, presenting the main data and the results and conclusions of 4 years of expts. on the nutritive values of treated and untreated milled rice, rice germ, bran and polish. Values are presented for protein efficiencies, for amino acid, vitamin, and mineral contents, and for supplementary values of added lysine, threonine and vitamin B_{12}. Treated (parboiled) rice showed higher nutritive values than untreated milled rice. The high nutritive value of rice germ suggests its use as an additive to human and animal foods.

Evolution of grain drying.

HUKILL, W. V. (Iowa Agr. Expt. Sta., Ames, Iowa). *Agr. Eng. 38*, 526–7 (1957); *Rice J. 60*, 18 (Dec. 1957).

A brief summary of developments in grain drying from initial studies around 1927, through use of drying generally for hybrid corn, and the rapid increase in grain drying after World War II. Discussion of this recent evolution divides the processes into fast and slow drying, or use of heated or unheated air. Various investigations of still unused methods are noted. Other topics include cooling after drying, effects of moisture migration in the grain, and the types of construction and of air-heating and circulating devices.

Fruits and Vegetables

Technology of fruit juices. II. Polarographic determination of the oxygen content of fruit juices.

MONZINI, A., BOTTALLA, G. AND DAGHETTA, A. (Univ. Milan). *Chimica (Milan) 33*, 157–60 (1957); *Chem. Abstr. 51*, 18376 d (1957).

The app. is described in detail. Polarographic diagrams were prepd. of the juice of pears, peaches, apricots, and oranges. The juices had a noticeable O content immediately after sealing in cans or in bottles, but after aging, it decreased to less than 0.02 ml./100 ml. juice which is the limit of sensitivity of the method.

Use of γ-radiation for preventing the sprouting of potatoes.

BURTON, W. G. AND HANNAN, R. S. (Ditton Laboratory and Low Temp. Research Sta., Dept. of Sci. and Ind. Research). *J. Sci. Food Agr. 8*, 707–14 (1957).

Irradiation at doses as low as 3500 r suppresses the sprouting of potatoes but it may also have harmful effects. After storage for 6 months or more, sweetening rendered the tubers unsuitable for crisp or chip production. After 15 months, a great accumulation of sugar and complete breakdown have been observed. Irradiation at 20,500 r and over increased the initial rate of H_2O loss and subsequent wilting. Irrespective of irradiation, there was an apparent direct correlation between the rate of H_2O loss and the cumulative loss which had occurred.

Milk and Milk Products

Preliminary experiments on the digestibility and on the biological value of the proteins of various kinds of cheese.

RANDOIN, L. AND CAUSERET, J. (Ann. Inst. nat. Rech. agron., Paris). *Ser. E, Ann. Tech. agric. 5*, 527–37 (1956); *Food Sci. Abstr. 29*, No. 1320 (1957).

The proteins of cheese have been found to be better digested by rats than the proteins of milk, but to have a lower biol. value. Observations on milk, drained curd, whey, and Camembert cheese showed that the proteins of curd and of cheese resembled each other in digestibility and in biol. value, and that the addn. of whey to the curd raised the biol. value markedly, although not always to the same level as that of milk. These results support the hypothesis that the lowered biol. value of cheese proteins, as compared with milk proteins, is due to loss of sol. proteins in the whey.

PACKAGING RESEARCH

Permeability of plastic bottle materials.

NIELSON, A. R. AND TEMPLE, E. J. (Plax Corp., Hartford, Conn.). *Package Eng. 3*, 21–6; 54 (Jan. 1958).

Blown plastic bottles made of 10 different polymers (5 polyethylenes, 2 nylons, 2 vinyls and 1 chlorinated polyether) were filled with 12 liquids (H_2O and 11 org. materials). Weight losses were measured during storage of the filled bottles at 73 and 120° F. Among the polyethylenes, higher-density resins had lowest permeability. Polar liquids such as alc. and H_2O in polyethylene bottles showed lower permeability than non-polar, as turpentine. Aromatic hydrocarbons permeated faster than aliphatics. Nylon, vinyl and polyether bottles were more resistant than any of the polyethylenes to non-polar organics and aromatics.

RADIOCHEMICAL TECHNOLOGY

Peripheral blood of the X-irradiated rhesus monkey.

RIOPELLE, A. J., ADES, H. W. AND MORGAN, F. E. (Emory Univ., Georgia). *Radiation Research 7*, 581–90 (1957).

Three groups of 4 rhesus monkeys each were irradiated with 350 r, 1000 r, or 2000 r by means of a Westinghouse 200-kv therapy X-ray machine. The latter exposures were fractionated into 10 exposures delivered at 2-week intervals. In general, the lymphocytes and neutrophils showed the pattern characteristic of other species after a single exposure. Recovery rates were slower after the multiple-dose schedule. All white blood counts were reduced 1½ years after the initial irradiation. Values for hemoglobin concn. were the least altered by the radiation exposures. The blood picture of an atypical individual was presented.

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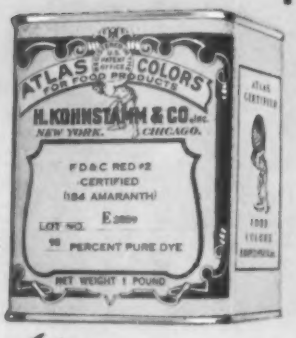

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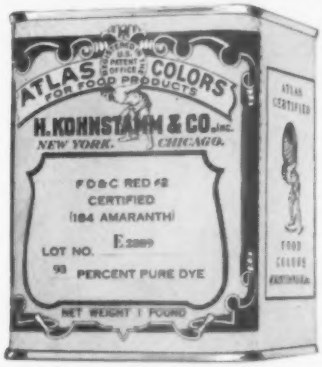

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PERSONNEL

Two new positions have been filled recently by the A. E. Staley Mfg. Co.

Dr. David P. Langlois has been named technical information scientist. Dr. Langlois has been leader of the company's carbohydrate research group since 1947. In his new assignment he will review and evaluate published research data from this country and abroad for information leading to the formulation of new research goals.

Dr. Langlois joined Staley's as a senior research chemist after receiving his doctorate from Pennsylvania State College in 1932.

Dr. D. P. Langlois

R. L. Nagle

Richard L. Nagle is now industrial marketing manager for the firm, a new position. The announcement came from L. S. Roehm, IFT member, who is vice-president and corn division manager. He said the new department will develop marketing programs and sales plans for the industrial products division.

Mr. Nagle has been industrial sales manager since 1952 and has been with the company for 18 years, starting as a salesman for retail package products in Georgia.

Two assignments to the technical sales staff have been announced by Firmenich, Inc.

R. E. Horrobin

C. R. Myers

Robert E. Horrobin, a member of IFT, has been assigned to the technical sales staff of the home office in New York City. He formerly was assistant manager of the Chicago office.

At the same time it was revealed that **C. Richard Myers** has been assigned to the technical sales staff in the Chicago office. Mr. Myers, also a member of the IFT, was formerly with Sterwin Chemicals Inc. and with the American Meat Institute Foundation.

Dr. Bernard L. Oser, IFT member, is now president of Food and Drug Research Laboratories, Inc. Dr. Oser was previously vice-president and director of the Laboratories, and in his new position he will continue to function as director.

Dr. B. L. Oser

Dr. Philip B. Hawk, who founded the organization, and who with Dr. Oser is a co-author of the standard text Practical Physiological Chemistry, has retired and is living in Florida.

E. R. Huckman

E. R. Huckman has been named as assistant field sales manager of the Foxboro Co. according to a recent announcement. With field sales manager **J. J. Burnett,** Mr. Huckman will coordinate sales engineering activities of field personnel serving instrument users in the northern and eastern sales regions.

Although Mr. Huckman will continue as regional manager of the New York area, his duties as manager of the New York City branch have been assumed by **H. R. Cushman,** formerly sales engineer at New York. Mr. Huckman is a chemical engineering graduate of MIT and has been with Foxboro since 1927.

Dr. Gerhard J. Fonken, a recent Ph.D. graduate from the University of California, has joined the research staff at Procter & Gamble's Miami Valley laboratories.

Thomas L. Murphy has been named vice president in charge of sales of the Aula division of Archibald & Kendall, Inc. Mr. Murphy has been associated with the firm

Sales representatives of H. Kohnstamm & Co., Inc., held their annual meeting recently in New York City. New products were introduced and uses discussed by the firm's technical staff. Those in the picture include: seated left to right, E. M. Moss, Louis J. Woolf, chairman of the board; Paul Kohnstamm, president; Arthur D. Vogel, vice president; Dr. Samuel Zuckerman, vice president; Dr. David Jorysch, an IFT member, assistant vice president. Next row, left to right, William J. Lane, Howard Zimmer, V. N. Tinnes, Andrew J. Torter, Si Slovitt, Michael Dunne, William E. Olson, Joseph P. Torter, Louis Agre, Carroll D. Biddison, Jesse White, Herb Johnson. Back row, left to right are Robert S. Woolf, Dr. Robert Cooney, assistant vice president; Jim McNellis, J. A. Holderreid, William J. Kelley, Harold Anderson, and G. H. Sloane.

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for 10 years, starting as a salesman, and since 1952, has been sales manager of the Aula division.

Dr. G. W. Bierman

Dr. George W. Bierman has recently joined the staff of Herbert V. Shuster, Inc., Food Consultants. Dr. Bierman, a member of IFT, will take over the duties of technical director. He was formerly associated with Friend Brothers, Inc., in the same capacity.

H. A. Levy

Howard A. Levy, in charge of Chicago sales for The Griffith Laboratories, Inc., celebrated his 25th anniversary with the company in January. Mr. Levy, a member of IFT, entered the service of the Griffith organization in the purchasing department. He entered sales about 23 years ago.

R. E. Moser

Roy E. Moser, Jr., an IFT member, has been named as extension fruit and vegetable processing specialist at Oregon State College to help commercial fruit and vegetable processors in Oregon to receive the latest information on new products and improved processing methods.

Mr. Moser who has a master's degree in food technology from the University of Massachusetts, has been a food technologist with the poultry branch of the U. S. Department of Agriculture and was extension food technologist with VPI for eight years before moving to Oregon.

Dr. C. B. Hartley

Dr. Clell B. Hartley, IFT member, has been appointed quality control manager for Fairmont Foods Co. He joined the firm's general office staff in Omaha as research laboratory manager a year ago.

In his new position, Dr. Hartley will be responsible for all quality control activities throughout the firm's 32-state manufacturing and distributing area. He holds his doctorate degree in dairy bacteriology and manufacturing from the University of Minnesota.

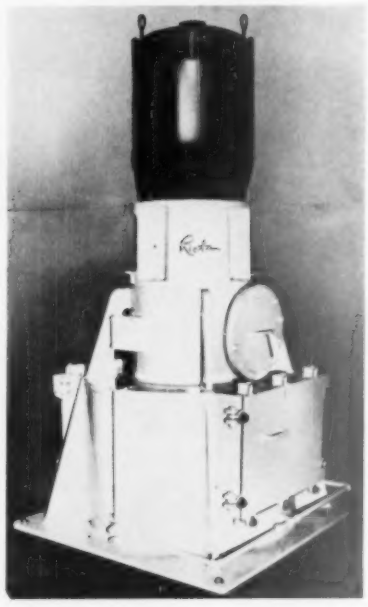
Dr. Max S. Dunn, professor of chemistry and physiological chemistry at the University of California at Los Angeles, has been appointed to the Research Advisory Board of Truesdail Laboratories, west coast independent research organization. Dr. Dunn, widely known for his pioneer work in amino acid and protein studies as well as research on metabolism antagonisms, will be called upon when confidential research for industrial clients requires highly specialized knowledge in this particular biochemical field.

J. P. Bainbridge

John P. Bainbridge, Jr., formerly associated with Monsanto Chemical Co., has joined Penick & Ford, Ltd., Inc. as assistant sales service manager to **Dr. J. E. Killinger.** His headquarters will be at Cedar Rapids, Iowa.

Mr. Bainbridge graduated as a chemical engineer from MIT in 1936 and joined Monsanto immediately. He has worked in the development and sales of chemicals to the paper, textile, leather and metal treating industries.

EQUIPMENT



In the grape industry it is said by the firm that the disintegrator is currently processing whole grapes including stems at the rate of 400 tons per hour. It is also used for large volume, continuous crushing or pulping in the processing of fruits, vegetables and citrus by-products.

PRODUCTS

Dodge & Olcott, Inc., 180 Varick St., New York 14, N.Y. is offering **"Spisorama Nutmeg,"** a dry, soluble product containing all the active flavoring elements of natural nutmeg, the company claims. It is said that the product is distilled from native spice and prepared on a dextrose base. Price ranges from $1.19 a pound in 200-pound lots to 99c a pound in 1000-pound lots.

A new **aluminum foil bread wrap,** said to be competitive in price with many conventional wraps has been developed by the research laboratories of Reynolds Metals Co. The new wrap, trademarked "Wrapseal," is a heat-sealable lamination of foil, paper, and wax. It is said to be able to provide maximum protection of flavor and freshness in all types of bread, both on the retail shelf and after it has been opened and reclosed in the home.

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